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BIOATCTIVITY OF PHLORETIN FROM *SYZGIUM POLYANTHUM* (WIGHT) WALP AS A GOUTY ARTHRITIS TREATMENT BASED ON SCREENING IN SILICO

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Abstract

Gouty Arthritis is a disease caused by the accumulation of monosodium urate in the joints due to hyperuricemia, which causes painful inflammation. *Syzgium polyanthum* (Wight) Walp is a plant that grows in Indonesia and has been used as a traditional medicine. It is used to treat various diseases, especially excessive production of uric acid. It is thought to contain phloretin compounds which can reduce the amount of uric acid. The purpose of this study was observed the biological activity of the natural products phloretin as an antihyperuricemia. Bioinformatic applications used in this study include the Pubchem compound database, protein target data base (Pharmmapper and Swiss Target Prediction), molecular docking software PyRx 0.8, software for 3D visualization and analysis of docking ligands and binding sites PyMOL and BIOVIA Disvovery Studo Client 2016. The results of docking and binding site analysis showed that phloretin was able to interact with cyclin dependent kinase 2 (target protein) with a binding affinity of -6 kcal/mol more negatively than allopurinol with a binding affinity of -5.7, at different binding sites. Phloretin is a potential compound that can be used in the treatment of gouthy arthritis as an anti-inflammatory by inhibiting CDK2 activity.

Key words: Gouty Arthritis, Phloretin, Screening In Silico, *Syzgium polyanthum* (Wight) Walp,.

INTRODUCTION

Uric acid (UA) mainly synthesis from liver and several in the intestine is the final product of purine metabolism in human (Yin et. al., 2022). Purine nucleotide by the action of adenosine deaminase produce adenosine, inosine and guanosine, adenosine is deaminated into inosine, inosine and guanosine are converted into hypoxanthine and guanine, hypoxanthine is converted to xanthine under the action of xanthine oxidase and guanine deaminates into xanthine. Xanthine is oxidized again by xanthine oxidase and inally produce uric acid (Álvarez & Macarrón, 2022; Keenan, 2020). UA in most of mammalian can oxidatively degradate into soluble compound allantoin (Wu et. al., 1989).

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The level of UA can be trouble when it is over condition exceeding 6.8mg/dL (Hyperuricemia) (George, 2023). Gouth arthritis (GA) is one of desease triggered by hiperuricemia. GA is an inflammation that occurs in the joints due to the accumulation of monosodium urate crystals which is triggered by increased levels of uric acid (Richette et. al., 2017; Yin, et.al, 2022). Inflammation that occurs in the joints triggers the secretion of several inflammatory factors such as cytokines and neutrophils recruiting into the joints (Zahroh & Faiza, 2018). Inflammation that occurs will cause symptoms of unbearable pain, swelling, and a feeling of heat in the joints). The secondary effects of GA are hypertension, atherosclerosis, insulin resistance, diabetes, liver and kidney disorders (Hayden, 2004; Hou et. al., 2012; So & Bernard, 2010).

Allopurinol is a drug commonly used by gout arthritis patients that action to reduce uric acid formation. Allopurinol is known to be a substrate of xanthine oxidase (Muhtadi, et. al., 2012). The use of allopurinol has several side effects such as diarrhea, nausea, and an increase in phosphate alkaline. Long-term use can cause allopurinol hypersensitivity syndrome (AHS), which is usually associated with an increased risk of poor kidney function (Stamp et. al., 2012).

Syzgium polyanthum (Wight) Walp is one of plant that grown in Indonesia and has been used as a traditional medicine. *S. polyanthum* (Wight) Walp is also known as salam, serai kayu, or samak (Malaysians), while in Indonesia it is known as umbai serai, meselengan, manting, Indonesian laurel or Indonesian bay leaf (Widyawati, et. al.., 2015). The leaves, fruit and bark of this plant are traditionally used for various medical and non-medical purposes. The roots and fruit are consumed to reduce the effects of drunkenness due to alcohol, while the leaves are traditionally consumed to treat various diseases, such as diabetes mellitus, hypertension, gastritis, ulcers, diarrhea, skin disease, and also various infections (Lelono et. al., 2009). S*. polyanthum* (Wight) Walp is rich in potency of pharmacological, including anti-cholesterol, anti-tumor, anti-diabetic, anti-microbial, anti-cancer, anti-oxidant, and anti-inflammatory (Ismail and Ahmad, 2019).

Syzgium polyanthum (Wight) Walp is contain of several phytochemical compound like carbohydrates, tannins, alkaloids, steroids, triterpenoids, and flavonoids from that leaf and unripe fruits. On fruit ripe contain saponins, carbohydrates, tannins,alkaloids, triterpenoids, and flavonoid (Kusuma et, al., 2011). Lelono et al. 2019 found that methanolic–water extract from S. polyanthum bark had the phenolic compound.

Phloretin ia the one of phenolic compound (Mariadoss et. al., 2019) is contained in S. polyanthum (Muhtadi et. al., 2012). Phloretin, a natural phenolic compound, is a dihydrochalcone characterized by the presence of 2,6-dihydroxyacetophenone pharmacophore. It is a versatile molecule with anticancer, anti-osteoclastogenic, antifungal, antiviral, anti-inflammatory, antibacterial and estrogenic activities and able to increase the fluidity of biological membranes and penetration of administered drugs (Behzad et. al., 2017). The phloretin in the Bay leaves are thought can reduce the amount of uric acid and reduce inflammatory response due to the accumulation of uric acid in the joints (Liu et. al., 2017).

The purpose of this study was to determine the bioactivity of phloretin compounds contained in S. polyanthum (Wight) for the treatment of gouty arthritis based on screening in silico.

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RESEARCH METHODS

Ligand Preparation

The 3D structure and SMILES of the ligand were obtained from pubchem compound database (https://pubchem.ncbi.nlm.nih.gov), phloretin with CID 4788 and phloretin eith CID 135401907

Selection of Target Protein

The target protein for Phloretin was obtained by entering SMILES into servers Swiss Target Prediction (http://www.swisstargetprediction.ch/) and Pharmmaapper (http://59.78.96.61/pharmmapper/submit_file.php). Servers will provide a list of predicted targets for phloretin compounds. The list of protein targets from these servers was compared and the most potential protein targets were taken according to references.

Molecular Docking

Molecular docking for phloretin dan allopurinol compounds and target proteins was carried out using PyRx 0.8 software.

Molecule Visualization And Intermolecular Interactions/ Force

Interactions between phloretin, target proteins and control compounds, visualized and analyzed using PyMoland and Discovery Studio 2016 Client.

RESULTS AND DISCUSSION

The result of protein target selection using pharmmapper (job ID: 170320111737) and swiss target prediction (SMILES: C1=CC(=CC=C1CCC(=O)C2=C(C=C(C=C2O)O)O)O) it was found that the protein target that had the most potential to interact with phloretin was cyclin dependent kinase 2 (CDK2). CDK2 as a one type of CDK is a protein kinase characterized by needing a separate subunit - a cyclin - that provides domains essential for enzymatic activity (Malumbers, 2014). CDK2 is one of the essential protein receptor targets in the treatment of Gouty Arthritis (Fan et. al. 2021) due to its role in regulating the inflammatory process (Hsu et.al., 2019).

Visualization results using PyMOL software show that Phloretin and Allopurinol compounds have the same site (Figure 1). Phloretin and Allopurinol were then tested with PyRx 0.8 Software used to perform molecular docking. Docking is a process in both molecules are matched by docking in 3D space. Molecular docking is used to determine the interaction between the ligands (phloretin and allopurinol) and the receptor (CDK2) as a target protein. This interaction is related to the position of the site binding and the strength of the bond between the ligand and the receptor.

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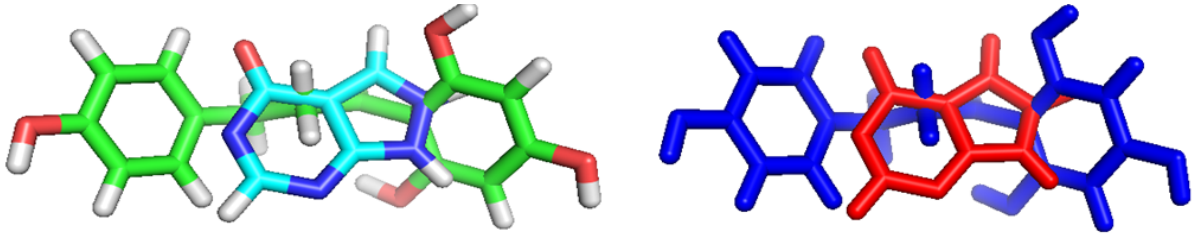

Figure 1. Visualization of Phloretin Compounds (Blue) and Allopuriol Compounds (Red) Using PyMOL Before Mollecular Docking was carried out, it was shown that both compounds were in the same site.

The docking results showed that both ligand compounds were able to interact and bind to the target protein receptor (CDK2), but with different binding site positions (Figure 2). The docking results also show binding affinity between phloretin and allopurinol with CDK2. Binding affinity phloretin with CDK the highest is at -6 and allopurinol with CDK at -5.7. Phloretin has a lower binding affinity than Allopurinol. It means, phloretin has a higher power or strength binding than allopurinol. The docking results were then visualized using the PyMOL software. It also showed that the phloretin and allopurinol compounds were able to interact and bind to CDK 2 with different binding sites.

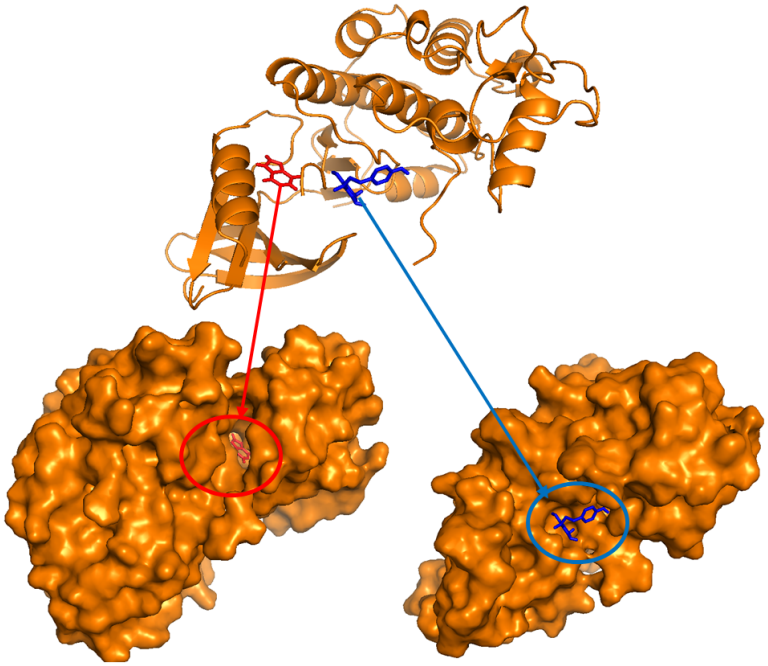

Figure 2. Docking Interaction Results Visualization between the target protein (Cyclin Dependent Kinase 2) and ligand compounds as inhibitors (phloretin and Allopurinol) shows that the ligand compounds are able to interact with the target protein, but with different binding sites. Description: orange (Cyclin Dependent Kinase 2), blue (phloretin), red (allopurinol).

BIOVIA Discovery Studio Client 2016 used to determine and show the type of bond and the location of the binding site of the ligand compound on the target protein. The visualization results show that phloretin interacts with CDK 2 by hydrogen bond, carbon-hydrogen bond, pi-donor hydrogen bond,

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interaction pi-pi T shaped, and Pi-Alkyl of the amino acids Tyr 15, Leu 148, and Arg 126 protein CDK 2. Amino acids Tyr 15 for hydrogen bond and , pi-donor hydrogen bond, Leu 148 for carbon-hydrogen bond, Agr 126 for Pi-Alkyl and the interaction pi-pi T shaped. Interaction distance between phloretin and Tyr 15 is 2.35 for hydrogen bonds, 3.18 for Pi-Donor hydrogen bond, 5.57 pi-pi T shaped; Leu 148 is 3.45, and Arg 126 is 4.57 (Figure 3).

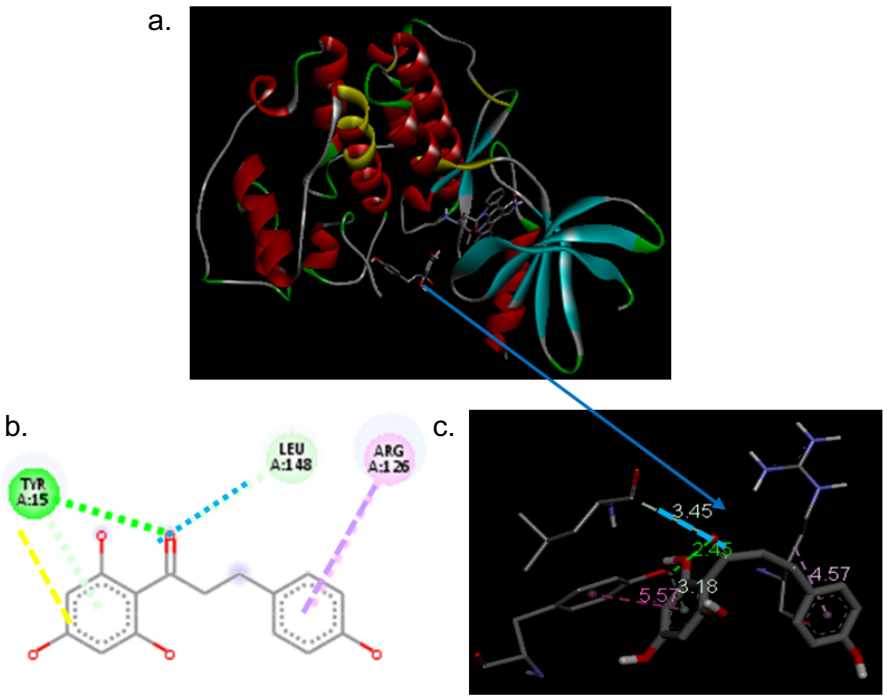

Figure 3. Visualization of Phloretin Interaction with Cyclin Dependent Kinase 2 (CDK 2) using BIOVIA Discovery Studio Client 2016.(a) 3D structure of Phloretin interaction with CDK 2.(b) Types of interactions between Phloretin and amino acids from CDK 2.(c) The distance between each type of interaction or bond between phloretin and amino acids CDK 2. Description: hydrogen bond (green dashed line), carbon-hydrogen bond (blue dashed line), pi-donor hydrogen bond (yellow dashed line), Pi-Alkyl interactions (purple dashed line), and pi-pi T shaped (pink dashed line) are not shown in figure b

Phloretin is thought to be able to bind strongly to CDK2, because CDK2 has a role in regulating inflammatory processes (Hsu et.al., 2019) including inflammation that occurs in gouty arthritis. CDK is able to regulate the secretion of inflammatory cytokines factors in macrophages (Laphanuwat & Jirawatnotai, 2019). Therefore, through the mechanism of inhibition of CDK2 activation, the chemotaxis of primary neutrophils will be reduced to reduce the inflammation that occurs (Hsu et.al., 2019). Phloretin is also capable of inhibited the activation of extracellular regulated protein kinases/nuclear factor-kappa B pathway which can reduce the expression of inflammatory factors and endothelial injury induced by high uric acid levels (Liu et. al., 2017).

Allopurinol interacts with CDK 2 by hydrogen bonds from the amino acids Val 64, Leu 143, Phe 146, and Asp 145 (Figure 4). The interaction distance between allopurinol hydrogen bonds with Val 64 is 2.28

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and 2.74, Leu 143 is 2.78, Phe 146 is 2.97 and Asp 145 is 2.38. Allopurinol was action through the reduction of metabolism purine by the inhibited xanthin oxidase that has a role for converted hipoxanthin and xanthine to uric acid (Pacher, 2006). Therefor, mainly protein target of Allopurinol is xanthine oxidase. Meanwhile, phloretin has a inflammation reduce activity that occur of uric acid accumulation injoints by the inhibited protein kinase activity. This is thought to be the cause of the difference in the binding site of phloretin-CD2 and allopurinol-CDK2.

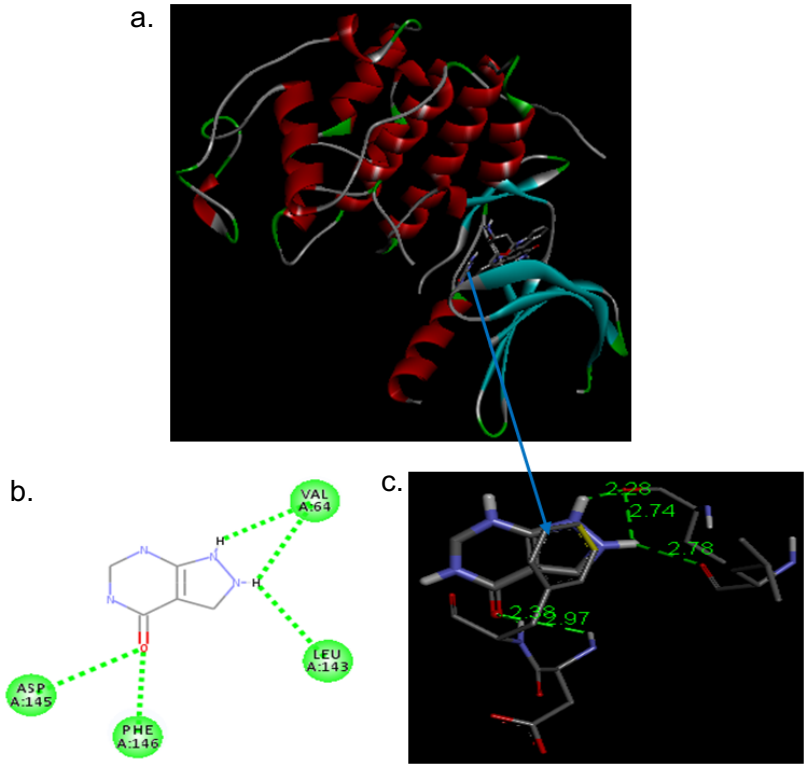

Figure 4. Visualization of Allopurinol Interaction with Cyclin Dependent Kinase 2 (CDK 2) using BIOVIA Discovery Studio Client 2016. (a) 3D structure of interaction of Allopurinol with CDK 2. (b) Types of interaction between Allopurinol and amino acids from CDK 2. (c) Range of types of interactions or bonds between phloretin and CDK 2 amino acids. Description: hydrogen bonds (green dashed line)

Phloretin also has activity to reduce uric acid levels through blocking of GLUT9-mediated UA uptake in human umbilical vein endothelial cells. GLUT 9 is one of 13 types of protein from the GLUT group (Glucose transporter) have a role as transporter that catalyzes the uptake of glucose from the blood circulation system into target tissue cells (Watson, 2001). GLUT 9 (SLC2A9) currently known not only as a fructose transporter but it is also known to have transporter activity for uric acid (Vitart et. al., 2008). GLUT 9 play a role in transport reabsorption of uric acid that it has an influence in determining the amount of uric acid in the body and through the mechanism of suppressing GLUT 9 activity can reduce the amount of uric acid (Hou et. al., 2012). In consequently, inhibition of GLUT 9 expression allows decreased activity of uric acid absorption, so that phloretin has the potential to reduce excessive amounts of the uric acid in

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various body tissues including joints.

DruLito software used for show the potential of compounds to be used as medicine. Lipinski's Rule of five is a program in DruLito used for used to evaluate druglikeness and determine the biological and pharmacological properties of a compound that has the potential as an active medicines that can given orally to humans. The results of the analysis of the DruLito software using Lipinski's Rule of five parameters, it was found that Phloretin has a 278.08 molecular weight, 1.803 lopP, 5 H-Bound acceptor, and 4 H-bond donor (Tabel 1.)

Table 1. Results of DruLito analysis using Lipinski's Rule of five parameters to determine the druglikeness of phloretin and allopurinol compounds

Lipinski's Rule / Compound	Molecular Weight	logP	H-Bond Acceptor	H-Bond Donor
Phloretin	274.08	1.803	5	4
Allopurinol	136.04	-0.64	5	2

Lipinski's Rule of five parameters indicate that a medicine compound will have poor absorption if it has more than 5 H-Bond donors, 10 H-Bonds acceptors, molecular weight (MWT) of more than 500 and calculation of Log P(CLogP) greater than 5 (or MlogP>4.15) (Lipinski et. al., 2001). Based on it, phloretin is a compound that has potentially as an antihyperuricemia medicine. This is because phloretin not only acts as an antihyperuricemia but also anti-inflammatory.

CONCLUSION

Phloretin is a potential natural compound that can be used as an anti-inflammatory in the treatment of gouty arthritis by inhibiting the CDK2 protein, based on its binding affinity) and intermolecular interactions. In silico screening between phloretin other protein targets such as GLUT9 is needed for further research in knowing more about the bioactivity of phloretin.

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