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Berberine: A Potential Inhibitor of Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS) for Malaria

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ABSTRACT

The goal of this study was to genetically link natural materials derived from Tinospora crispa L with Berberine to dihydrofolate reductase-thymidylate synthase (DHFR-TS). Method: The ligand berberine (CID: 2353) was obtained from Pubchem, while the protein DHFR-TS (PDB ID 2bl9) was obtained from Protein Data Bank. The ligands and proteins interacted with HEX 8.0.0.0 and were visualized with Discovery Studio. The researchers discovered a positive interaction between berberine and DHFR-TS, observed at four amino acid residues that bind to the protein TYR125, ILE121, LEU45, and MET54. Van der Waals interactions, hydrogen bonds, Pi-Sulfur, Pi-Alkyl, and Pi-Stalked interactions all contribute to strength and stability. In conclusion, berberine has the potential to act as a DHFR-TS inhibitor and thus prevent malaria.

Potensi Berberin sebagai anti-malaria melalui penghamabatan dihydrofolate reductase-thymidylate synthase (DHFR-TS) Malaria

ABSTRAK: *Tujuan penelitian ini adalah memanfaatkan bahan alam yang berasal dari Tinospora crispa L dengan kandungan utama Berberin dengan dihydrofolate reductase-thymidylate synthase (DHFR-TS) secara genetik. Metode, Ligan berberin (CID: 2353) diperoleh dari Pubchem sedangkan protein DHFR-TS (PDB ID 2bl9) diperoleh dari Protein Data Bank, ligan dan protein diinteraksikan menggunakan HEX 8.0.0.0 dan divisualisasikan menggunakan discovery studio. Ditemukan interaksi positif antara berberin dan DHFR-TS yang menunjukkan interaksi pada empat residu asam amino yang berikatan dengan protein. Mereka adalah TYR125, ILE121, LEU45 dan MET54. Beberapa interaksi yang dilakukan Van der Waals, ikatan hidrogen, Pi-Sulfur, Pi-Alkyl dan Pi-Stalked juga memberikan dukungan dalam rangka meningkatkan kekuatan dan stabilisasi. Kesimpulannya, berberin memiliki potensi fungsi sebagai penghambat DHFR-TS dan mengarah pada malaria.*

INTRODUCTION

Malaria is an infectious disease caused by a parasite and transmitted by the *Anopheles* Sp mosquito, which is endemic in eastern Indonesia (Cowell and Winzeler, 2019; Kojom Foko et al., 2019; Selasa, 2017; Willa and Mading, 2014). According to data on positive malaria cases and the number of malaria sufferers (Annual Parasite

Incidence/API), high endemic malaria remains concentrated in eastern Indonesia, particularly in the province of East Nusa Tenggara (M Taek et al., 2018; Taek et al., 2019). According to statistics, East Nusa Tenggara province had the third highest number of cases in 2014, with a high malaria case API rate (12.81%)(Purba et al., 2017). Artemisinin-based Combination Therapy is used in Ende Regency, which has a population of 260,988 people and an Annual Parasite Incidence of 23.20 (67.11%) (Purba et al., 2017).

In the past, synthetic drugs were used to treat malaria. Quinine has been used as a drug since its discovery in 1820. Mepacrine, chloroquine, and mefloquine are also used (Lu et al., 2018; Wang et al., 2020). Drug administration became less effective as the parasite's development and changes began to show signs of drug resistance. As a result, drug administration has been discontinued or limited to specific situations (Tse et al., 2019). Natural materials are expected to have potential in medicine, with the use of plant secondary metabolite derivatives serving a medical function (Schröter et al., 2019). Natural ingredients are expected to have fewer side effects and improve healing (Chaudhury et al., 2017; Kesuma et al., 2018).

Natural bioactive compounds in the brotowali plant (*Tinospora crispa*) include alkaloids, tinocrisposide, palmatine, berberine, kaolin, and columbine (Yesi et al., 2019). Tinospora crispa bioactives have antidiabetic, anti-inflammatory, and antimalarial properties (Malik, 2015). Tinospora crispa's theoretical study as an antimalarial must be documented with local knowledge before the data is scientifically tested using bioinformatics studies as a molecular biology method. The treatment is nutrigenomic, meaning nutrition is provided at the gene level. Genetic disorders are linked to malaria susceptibility or resistance, and heritability studies suggest that genetic factors account for approximately 25% of the risk of developing severe malaria (de Mendonça et al., 2012).

Over the last two decades, therapy against Plasmodium vivax parasites with the malaria drug chloroquine (CQ) has resulted in reports of a significant increase in Plasmodium vivax resistance to CQ (Poespoprodjo et al., 2009; Auliff et al., 2012; Imlay et al., 2015; Kojom Foko et al., 2019). Malaria enzymes involved in folate metabolism have been identified as potential antimalarial drug targets. This enzyme has also been validated as a target of dihydrofolate reductase-thymidylate synthase (DHFR-TS) (Luzz et al., 2001; Gibson et al., 2016). DHFR-TS1 and DHFR-TS2 are found on chromosomes 2 and 4 in areas where chromosomal duplication has occurred. The molecular masses of the DHFR-TS polypeptides differed slightly, with DHFR-TS1 and DHFR-TS3 being 55 and 57 kD, respectively, and DHFR-TS2 being 63 kD. The difference in molecular weight between DHFR-TS1 and DHFR-TS3 is caused by transit peptide content (Landau et al., 2013; Gorelova et al., 2017). This study aims to use berberine, a bioactive compound found in Tinospora crispa L, as an inhibitor of dihydrofolate reductase-thymidylate synthase (DHFR-TS) to treat malaria infection.

METHOD

In this research, the in silico method was used (Bare & Sari, 2021). The in silico method is a study that combines the use of informatics with the investigation of biological mechanisms. Secondary data is used in in-silico research, taking the three-dimensional structure of compounds and proteins from each database. The berberine ligand (CID: 2353) came from Pubchem, and the DHFR-TS protein (PDB ID 2bl9) came from the Protein Data Bank. HEX 8.0.0.0 software was then used to interact with the ligand and protein. The interaction of berberine with DHFR-TS was analyzed and visualized using the discovery studio software version 21.1.1. The parameters studied included amino acid residues interacting with berberine, bond types, Van

Der Waals forces, and physicochemical bond properties.

RESULTS AND DISCUSSION

Berberine's interaction with DHFTRS resulted in the binding of four amino acid residues, namely TYR125, MET54, LEU45, and ILE121 (Figure 1). These four amino acid residues form a variety of bonds, including carbon-hydrogen bonds (TYR125), pi-sulfur bonds (MET54), and alkyl pi-alkyl bonds (LEU45 and ILE121) (Figure 1). Van der Waals forces surround the Berberine-DHFTRS interaction at ten amino acid residues: ILE173, SER58, LEU128, PHE57, ARG131, PRO129, PRO122, GLY43, THR44, SER120 (Figure 1).

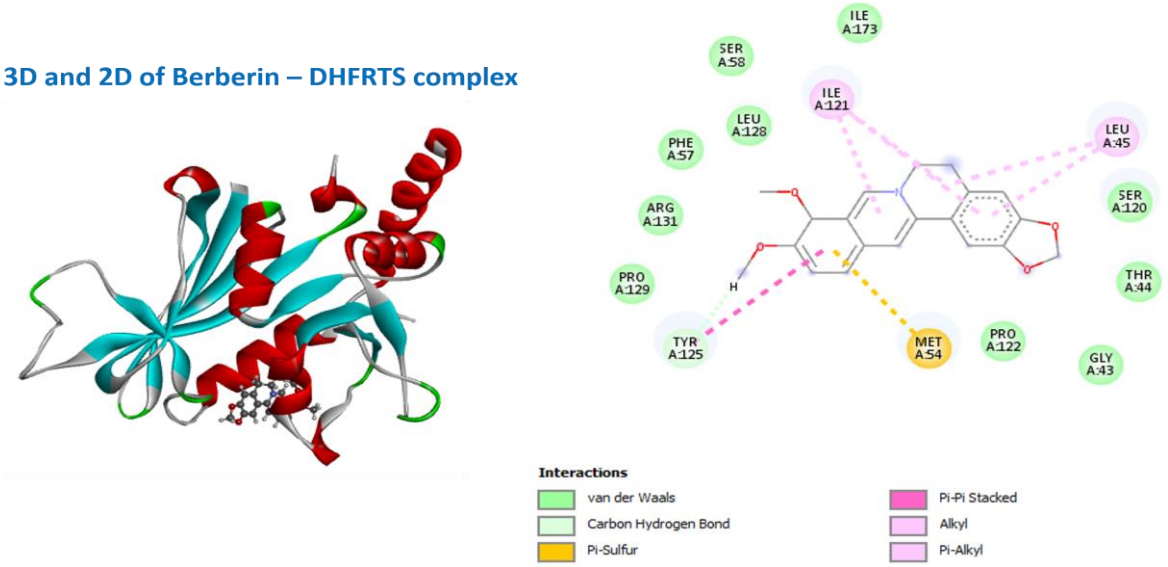

Figure 1. The 3D and 2D Interactions of Berberine Compounds against DHFTRS Protein

Because most of the surface is covered by grey, the interaction formed has a neutral state with a value of 0. (figures 2a, 2b, 2e). The hydrophobicity of berberine-DHFTRS is neutral, with the blue and grey sides complementing each other in the interaction (Figure 2c). In contrast, the amino acid residues appear as donors and acceptors to the DHFTRS ligand, with purple (donor) and green (acceptor) colours (Fig. figure 2f). Because most of the surfaces on the DHFTRS surface are covered in blue, the solvent-accessible surface (SAS) value is very high (figure 2e).

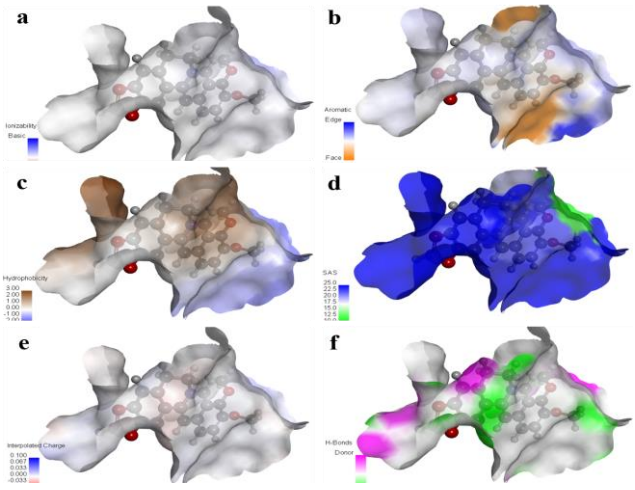

Figure 2. Physicochemical properties of Berberine-DHFTRS interaction. a. Ionizability b. Aromatic c. Hydrophobicity d. SAS e. Interpreted Charge f. H-Bonds

The formation of the formed bond energy is influenced by the number of hydrogen bonds, hydrophobic interactions, and Van Der Waals forces (Bare, 2021; Elfi et al., 2021). The lower the bond energy formed and the stronger the bond between the compound and protein, the more complex the compound with the interacting protein and the greater the number of bonds in the complex (Bare et al., 2019, 2020, 2021; Sari et al., 2020a, 2020b; Krisnamurti et al., 2021).

DHFR-TS is a target for malaria treatment, and this study found a molecular interaction between the compound berberine and DHFR-TS. In the case of pyrimethamine and cycloguanil, the inhibitory system on DHFR-TS (Yuvaniyama et al., 2003; Bare et al., 2022) is predicted to have structural similarities to natural folate, a class of antimetabolites known collectively as antifolates. This compound depletes the cellular THF pool, which inhibits dTMP and DNA synthesis, resulting in "thymineless death" (Gibson et al., 2016; Gorelova et al., 2017). Berberine's interaction in this study is predicted to be an antifolate agent that directly inhibits TS.

CONCLUSIONS AND SUGGESTIONS

The findings indicate that the berberine found in *Tinospora crispa* L has the potential to act as a DHFR-TS inhibitor, allowing it to be used as an alternative natural ingredient in malaria treatment. Further antimalarial testing will require in vitro and in vivo tests.

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