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THE IN SILICO STUDY: CURCUMIN POTENTIAL AS A TOPOISOMERASE ENZYME INHIBITOR IN THE REPLICATION PROCESS OF PLASMODIUM FALCIPARUM THAT CAUSES CEREBRAL MALARIA

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ABSTRACT

Turmeric (Curcuma longa) contains the active compound curcumin, which has antiprotozoal, antimalarial, anti-inflammatory effects. This study aims to ignite the potential of curcumin, which has the potential as an antiprotozoal through inhibition of Plasmodium Falciparim replication through the topoisomerase enzyme. The method used in this study is the One Shot Experimental Study, with several stages including preparation of active compounds, prediction of active substance binding energy, prediction of compound binding, molecular docking, prediction of ADME (absorption, distribution, metabolism, excretion) and toxicity. The results show that curcumin binds to the same site as Artemisinin, both also interact with the topoisomerase VI protein and provide similar inhibitory effects. ADME predictions show that curcumin has good potential for use as an oral drug, with both LD50s included in class 4. The binding affinity and bioactivity of curcumin are lower than Artemisinin but are still considered to have the potential as a safer antiprotozoal alternative.

KEYWORDS *Plasmodium falciparum, Curcuma longa, Topoisomerase enzyme, turmeric*

INTRODUCTION

Malaria is caused by protozoan parasites belonging to the genus Plasmodium. Malaria that infects humans can be caused by 5 types of Plasmodium, namely P. Falciparum, P. Malariae, P. Vivax, P. Ovale and P Knowlesi (Habibi et al., 2022). Cerebral malaria (MS) is the most severe and fatal neurological complication leading to death (Jain et al., 2013).

The World Health Organization (WHO) 2020 shows that 1.7 billion cases of malaria worldwide (Kogan & Kogan, 2020; Monroe et al., 2022). Indonesia is the second highest in Southeast Asia for the highest number of malaria cases (WHO, 2022). The highest cases in the eastern region are 400,253 cases in 2022, about 356,889 cases from Papua Province. About 80% of malaria deaths are caused by children under the age of

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five, who are the most likely group to contract the disease. (Indonesian Ministry of Health, 2022).

Malaria is transmitted to humans through the bite of a female Anopheles mosquito infected with Plasmodium parasites (Markwalter et al., 2024a, 2024b). In addition, other forms of malaria transmission can include transmission from a pregnant woman infected with malaria to her fetus, and transmission through blood transfusions contaminated with plasmodium parasites (CDC, 2023).

The first-line treatment option is Artemisinin, which provides a rapid and sustained parasitologic cure in patients with Plasmodium falciparum malaria and has been shown to reduce transmission in areas of low and moderate endemicity (van Der Pluijm et al., 2020). Potentially severe side effects include QTc interval prolongation, cardiac arrhythmias, liver damage. However, Therapeutic Efficacy Studies (TES) show an increase in drug resistance in malaria, where currently available antimalarial drugs such as artemisinin and chloroquine (NLM, 2017). Therefore, alternative treatments are needed, one of which is turmeric extract or Curcuma longa (Belay et al., 2024; Jawale, n.d.).

Curcumin, a natural polyphenol derived from rhizomatous perennial plants or turmeric, is proven as an antimalarial, anti-inflammatory, antioxidant, anticancer, and cardiovascular enhancement agent (Kumpitak et al., 2024). Curcumin contained in turmeric can prevent the activity of the topoisomerase enzyme, which is required for DNA replication. This enzyme may represent a potential selective target to be explored for drug development against malaria (Jamil et al., 2023).

In silico can be used in visualizing an experiment / trial that will be done with the help of a computer (Jabeen et al., 2024). This in silico test can be used to determine and predict the occurrence of an interaction between a compound and the desired target molecule and one of them is a receptor (Takken et al., 2024). The interaction of a compound with this receptor can be visualized using computational methods and can be used to determine the pharmacophore of the compound used (Setiawan et al., 2016).

Based on this description, researchers are interested in conducting research on In Silico Studies: Potential of Curcumin as a Topoisomerase Enzyme Inhibitor in the Replication process of Plasmodium Falciparum that causes Cerebral Malaria.

RESEARCH METHOD

This study uses a One Shot Experimental Study design, which aims to test the potential of active compounds in Turmeric (Curcuma longa) as topoisomerase enzyme inhibitors in Plasmodium falciparum. The research method used was In Silico study with molecular docking using Molegro virtual Docker 5.0 program. After that, the prediction of bio-activity as antiprotozoal on Way Two Drug Pass Online webserver (http://www.way2drug.com/passonline/), ADME prediction of compounds using SWISS ADME webserver (http:www.swissadme.ch/index.php) and toxicity prediction using Pro-Tox Webserver (http://tox.charite.de/protox_II/).

RESULT AND DISCUSSION

Predictive Analysis of Interaction of Curcumin and Artemisin Compounds with Topoisomerase Proteins

Table 1. Predicted Active Substance Bond Energy

No.	Compound	Bond energy (Kj/mol)
1.	Artemisinin	-185
2.	Curcumin	-363,6

The binding affinity value is used to predict the strength of an interaction between ligand and protein, the strength of this interaction can be known with the Molegro virtual Docker 5.0 program with a maximum Molecular surface van der Waals parameter of 5. The interaction between ligand and receptor is said to be stronger if the value obtained is increasingly negative. Curcumin has a binding affinity value of -363.6 Kj/mol, while Artemisinin -185, indicating that Curcumin binds more strongly to the receptor and its inhibitory effect is greater than Artemisinin.

Table 2. Binding Prediction Results of Curcumin and Artemisinin Compounds with Topoisomerase VI Protein

Ligand	Bond Energy	Interaction	Distance (A)	Bond Type	Bond Type
Artemisin	-185	A:THR111:OG1 - :10:O3	2.72357	Hydrogen Bond	Conventional Hydrogen Bond
		A:THR111:OG1 - :10:O5	2.76482	Hydrogen Bond	Conventional Hydrogen Bond
		A:THR111:CA - :10:O2	3.55573	Hydrogen Bond	Carbon Hydrogen Bond
		A:**LYS113**:CE - :10:O4	3.60269	Hydrogen Bond	Carbon Hydrogen Bond
		:10:H4 - A:LEU110:O	2.87654	Hydrogen Bond	Carbon Hydrogen Bond
		A:**LYS104** - :10	5.13343	Hydrophobic	Alkyl
		A:LEU126 - :10	4.09234	Hydrophobic	Alkyl
		:10:C12 - A:LEU126	3.67019	Hydrophobic	Alkyl
		:10:C12 - A:LYS127	4.66863	Hydrophobic	Alkyl
		:10:C15 - A:LYS104	4.41121	Hydrophobic	Alkyl
		A:PHE105 - :10	5.27589	Hydrophobic	Pi-Alkyl
		A:PHE105 - :10:C12	4.71271	Hydrophobic	Pi-Alkyl
		A:SER112:OG - :10:C15	1.98767	Unfavorable	Unfavorable Bump
		A:SER112:OG - :10:H22	1.2968	Unfavorable	Unfavorable Bump

Curcumin	-363,6	A:ASP101:N - :10:O3	3.39317	Hydrogen Bond	Conventional Hydrogen Bond
		A:**LYS104**:NZ - :10:O1	3.06265	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H13 - A:LYS98:O	1.92469	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H14 - A:ASP49:OD2	2.13295	Hydrogen Bond	Conventional Hydrogen Bond
		A:**LYS113**:CE - :10:O6	2.68726	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H16 - A:LYS98:O	3.00535	Hydrogen Bond	Conventional Hydrogen Bond
		:10:H20 - A:GLY96:O	2.87974	Hydrogen Bond	Conventional Hydrogen Bond
		A:ILE99:CG1 - :10	3.88111	Hydrophobic	Pi-Sigma
		:10:C20 - A:LYS98	5.39003	Hydrophobic	Alkyl
		:10:C21 - A:LYS98	4.94046	Hydrophobic	Alkyl
		:10:C21 - A:ILE99	4.84726	Hydrophobic	Alkyl
		:10 - A:**LYS104**	3.92722	Hydrophobic	Pi-Alkyl
		:10 - A:ALA44	5.30863	Hydrophobic	Pi-Alkyl
		:10 - A:LYS95	4.39263	Hydrophobic	Pi-Alkyl

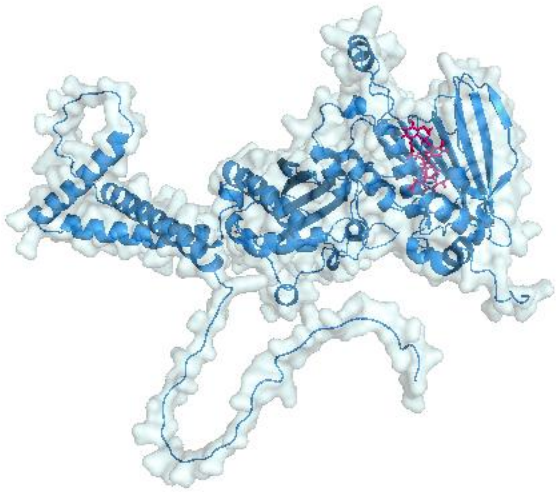

Figure 1. Visualization of the interaction of curcumin and artemisinin compounds with topoisomerase VI protein

Docking results with Molegro virtual docking version 5.0 were combined with proteins using PyMol software version 2.3 docking visualization to display 3D and 2D views and their interactions with Discovery Studio program version 21.1.1. Analysis to evaluate the interaction of curcumin and artemisinin compounds bind to topoisomerase VI protein. Shows the results that both form various types of bonds, including hydrogen

bonds, hydrophobic bonds, Conventional Hydrogen Bond, Carbon Hydrogen Bond, pi-sigma, alkyl, pi-alkyl, Unfavorable, Unfavorable Bump.

The types of bonds formed between curcumin and topoisomerase proteins are 7 hydrogen bonds and 7 hydrophobic interactions at residues ASP101, LYS104, LYS98, ASP49, LYS113, LYS98, GLY96, ILE99, ALA44, and LYS95. Interestingly, the topoisomerase residues LYS113 and LYS104, identified in curcumin were also present in artemisinin, indicating the same inhibitory mechanism as artemisinin against topoisomerase VI protein. This suggests that Artemisinin and curcumin can act as inhibitors of protein topoisomerase VI in its replication process.

ADME Analysis

Table 3. ADME Prediction Results of Artemisinin and Curcumin

Pharmacokinetic parameters			Artemisinin		Curcumin	
Category	**Name**	**Unit**	**Value**	**Confidence**	**Value**	**Confidence**
Absorption	Caco-2 (logPaap)	logPaap	-4,59	-	-4,5	-
	Human Oral Bioavailability 20%	Category (Bioavailable / Non-Bioavailable)	Bioavailable	0,866	Bioavailable	0,534
	Human Intestinal Absorption	Category (Absorbed/Non-Absorbed)	Absorbed	0,967	Absorbed	0,965
	Madin-Darby Canine Kidney	cm/s	-4,66	-	-4,74	-
	Human Oral Bioavailability 50%	Category (Bioavailable / Non-Bioavailable)	Non-Bioavailable	0,459	Bioavailable	0,703
	P-Glycoprotein Inhibitor	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,122	Inhibitors	0,94
	P-Glycoprotein Substrate	Category (Substrate/Non-Substrate)	Non-Substrate	0,086	Non-Substrate	0,15
	Skin Permeability	log Kp	-2,18	-	-2,3	-
Distribution	Blood-Brain Barrier (Central Nervous System)	log BB	-3,07	-	-2,61	-
	Blood-Brain Barrier	Category (Penetrating/ Non-Penetrating)	Penetrable	0,825	Non-Penetrable	0,049
	Fraction Unbound (Human)	free proportion	0,51	-	1,1	-
	Plasma Protein Binding	therapeutic index	11,68	-	36,08	-

	Steady State Volume of Distribution	log VDss	1,15	-	0,79	-
Metabolism	Breast Cancer Resistance Protein	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,149	Inhibitors	0,52
	CYP 1A2 Inhibitor	Category (Inhibitor/Non-Inhibitor)	Inhibitors	0,927	Inhibitors	0,665
	CYP 1A2_substrate	Category (Substrate/Non-Substrate)	Non-Substrate	0,327	Substrate	0,618
	CYP 2C19 Inhibitor	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,001	Inhibitors	0,846
	CYP 2C19_substrate	cyp2c19_substrate	Non-Substrate	0,348	Non-Substrate	0,407
	CYP 2C9 Inhibitor	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,005	Inhibitors	0,912
	CYP 2C9 Substrate	Category (Substrate/Non-Substrate)	Non-Substrate	0,004	Substrate	0,98
	CYP 2D6 Inhibitor	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0	Inhibitors	0,974
	CYP 2D6 Substrate	Category (Substrate/Non-Substrate)	Non-Substrate	0,4	Non-Substrate	0,347
	CYP 3A4 Inhibitor	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,043	Non-Inhibitors	0,499
	CYP 3A4 Substrate	Category (Substrate/Non-Substrate)	Substrate	0,839	Non-Substrate	0,15
	OATP1B1	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,041	Non-Inhibitors	0,324
	OATP1B3	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,064	Non-Inhibitors	0,114
Excretion	Clearance	Log (ml/min/kg)	14,87	-	6,14	-
	Organic Cation Transporter 2	Category (Inhibitor/Non-Inhibitor)	Non-Inhibitors	0,215	Non-Inhibitors	0,373
	Half-Life of Drug	Category (Half-life >=	Half-Life < 3hs	0,131	Half-Life < 3hs	0,464

	3hs/ Half-life < 3hs)

Absorption, Distribution, Metabolism, and Excretion (ADME) predictions that explain the flow of the compound's journey in its biological activity, starting from absorption until it reaches the target organ and produces a therapeutic effect. The human gut serves as the main organ where absorption of drugs administered via the oral route takes place. A compound is considered to have a good absorption rate if the absorption value is more than 80%, while it is considered to have poor absorption if the absorption value is less than 30%. Based on the research, the curcumin compound has a good small intestinal absorption value with an absorption percentage of 96.5%, almost the same as artemisinin with an absorption percentage of 96.7%.

A compound is considered to have a low volume of distribution if the distribution value is less than -0.15, and it is considered high if it is more than 0.45. In this case, the distribution value of curcumin (0.79) is lower than that of artemisinin (1.15). Therefore, it can be concluded that both can distribute themselves evenly in the body and are quite maximal in reaching concentrations similar to those in blood plasma.

The two major subtypes of cytochrome P450 enzymes are CYP2D6 and CYP3A4. The metabolic properties of curcumin and artemisinin are quite good as both compounds are able to metabolize CYP3A4 substrates. Thus, it can be concluded that both compounds are likely to be metabolized by P450 enzymes.

The excretion process of a compound can be anticipated by measuring the Total Clearance value and whether the compound is a substrate of the Renal Organic Cation Transporter 2 (OCT2).The excretion value of the clearance of curcumin is lower than that of artemisinin. From both values, it can be expected that curcumin compounds will be excreted more slowly than artemisinin.

Lipinski's Rules Of Five Analysis

Table 4. Lipinski's Rules Of Five Analysis

Description	Artemisin	Curcumin
Molecular Weight (g/mol)	**262.30**	**368.38**
Hydrogen Bond Acceptors	**4**	**6**
Hydrogen Bond Donors	**1**	**2**
Molar Refraction	**69.32**	**102.80**
Lipophilicity	**<5**	**<5**
Number of Atoms	19	27
Water Solubility	Soluble	Moderately soluble
Gi Absorption	High	High
BBB Permeant	Yes	No.
Bioavaibility	0.55	0.55

Lipinski's Rule of five:

1. <5 *hydrogen bond donors*
2. <10 *hydrogen bond acceptors*
3. *Mollecular weight* < 500 daltons
4. <5 *lipophilicity*
5. *Molar refractivity* (40-130)

This study shows Artemisinin and Curcumin as oral drugs based on Lipinski's Rule of five, both qualify as candidates for oral drug development.

Toxicity Analysis

Table 5. Toxicity Analysis of Target Compounds on Organs

Classification	Target	Artemisin	Curcumin
Target Organ	Hepatotoxicity	0.61	0.61
	Neurotoxicity	0.76	0.81
	Nephrotoxicity	0.50	0.59
	Respiratory toxicity	0.82	0.77
	Cardiotoxicity	0.78	0.55
Toxicity end points	Carcinogenecity	0.55	0.84
	Immunotoxicity	0.96	0.92
	Mutagenecity	0.77	0.88
	Cytotoxicity	0.84	0.88
	BBB-barrier	0.91	0.58
	ecotoxicity	0.59	0.75
	Clinical toxicity	0.55	0.61
	Nutritional toxicity	0.75	0.74
Tox21-nuclear receptor signaling pathways	Aryl hydrocarbon Receptor (AhR)	0.97	0.54
	Androgen Receptor (AR)	0.78	0.99
	Androgen Receptor Ligand Binding Domain (AR-LBD)	0.76	0.99
	Aromatase	0.71	0.90
	Estrogen Receptor Alpha (ER)	0.85	0.51
	Estrogen Receptor Ligand Binding Domain (ER-LBD)	0.93	0.83
	Peroxisome Proliferator Activated Receptor Gamma (PPAR-Gamma)	0.94	1.0
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	0.93	1.0
	Heat shock factor response element (HSE)	0.93	1.0
	Mitochondrial Membrane Potential (MMP)	0.70	1.0
	Phosphoprotein (Tumor Suppressor) p53	0.82	1.0
	ATPase family AAA domain-containing protein 5 (ATAD5)	0.81	0.94
Molecular Initiating Events	Thyroid hormone receptor alpha (THRα)	0.73	0.90
	Thyroid hormone receptor beta (THRβ)	0.93	0.78
	Transtyretrin (TTR)	0.55	0.97

	Ryanodine receptor (RYR)	0.89	0.98
	GABA receptor (GABAR)	0.53	0.96
	Glutamate N-methyl-D-aspartate receptor (NMDAR)	0.99	0.92
	alpha-amino-3-hydroxy-5- methyl-4-isoxazolepropionate receptor (AMPAR)	1.0	0.97
	Kainate receptor (KAR)	1.0	0.99
	Achetylcholinesterase (AChE)	0.52	0.61
	Constitutive androstane receptor (CAR)	1.0	0.98
	Pregnane X receptor (PXR)	0.74	0.92
	NADH-quinone oxidoreductase (NADHOX)	0.67	0.97
	Voltage gated sodium channel (VGSC)	0.94	0.95
	Na+/I- symporter (NIS)	0.82	0.98
Metabolism	Cytochrome CYP1A2	0.90	0.78
	Cytochrome CYP2C19	0.94	0.94
	Cytochrome CYP2C9	0.74	0.89
	Cytochrome CYP2D6	0.89	0.81
	Cytochrome CYP3A4	0.75	0.63
	Cytochrome CYP2E1	0.99	1.0

The results showed that artemisinin has respiratory toxicity, cardiotoxicity to target organs while curcumin is considered safer in its toxic effects on target organs. On toxicity end points, artemisinin showed immune, BBB, and nutritional toxicity. Tox21-nuclear receptor signaling pathways curcumin is more dominant toxic than Artemisinin.

Table 6 Toxicity Prediction Results of Targeted Compounds

Parameters	**Artemisinin**	**Curcumin**
LD50	900 mg/kg	2000mg/kg
Toxicity Class	4	4

In this study, Artemisinin and curcumin have LD50 values of 900 mg/kg and 2000 mg/kg, respectively, belonging to toxicity class 4, which indicates the potential danger when ingested in large quantities with different doses.

Bioactivity Analysis as Antiprotozoal

Table 7. Bioactivity of Target Compounds

Bioactivity	Artemisin	Curcumin
Antiprotozoal	0.992 Pa	0.232 Pa
Antiprotozoal (Plasmodium)	0.954 Pa	0.160 Pa

PASS prediction results are interpreted and used flexibly: (1) If pa>0.7, the chance of finding activity experimentally is high; (2) If 0.5<pa<0.7, the chance of finding activity experimentally is smaller, but the compound may not be so similar to known

pharmaceutical agents; (3) If pa<0.5, the chance of finding activity experimentally is smaller, but the chance of finding new compounds structurally is high. (Goel *et al.*, 2011)

In this study, the authors wanted to determine the biological activity of curcumin compounds specifically as antimalarial and compare with known pharmacological agents (contro), namely Artemisin. From the results obtained, artemisin has antiprotozoal activity of 0.954Pa, while curcumin has activity as an antiprotozoal of 0.160 Pa. The analysis of this study shows that curcumin has lower antiprotozoal activity than Artemisin.

CONCLUSION

The conclusion of this study shows that curcumin, the active compound in turmeric or *Curcuma longa* has potential as an antiprotozoal against the mechanism of inhibition on the topoisomerase enzyme *Plasmodium falciparum.* Although the antiprotozoal bioactivity of curcumin is lower than Artemisinin, curcumin has the opportunity to find structurally new compounds. Curcumin fulfills Lipinski's rule of five criteria with better ADME to be developed as a safe and efficient drug candidate. The LD50 of curcumin is high compared to Artemisinin as a control, so it is considered safer to use.

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