Antiviral Activitiy of Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin and Cyclocurcumin compounds of *Curcuma longa* against NSP3 on SARS-CoV-2

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Abstract

SARS-CoV-2 genome encodes two large polyproteins (pp), pp1a and pp1ab which are cleaved and transformed into a mature form by a protease, non-structural protein 3 (NSP3). NSP3 is encoded by open reading frame (ORF) 1a/b. Curcuma longa (*C. longa*) or turmeric has been documented to have antiviral effects. The aim of this study was to assess the viral activities of *C. longa* against SARS-CoV-2 focusing on its potency to inhibit viral replication by targeting NSP3. PubChem databases were used to obtain the metabolic profile of *C. longa*. The compound's interaction with nucleocapsid was analyzed using molecular docking with Molegro Virtual Docker. Bioinformatics analysis based on rerank score presents all compounds of *C. longa* have higher binding affinity than the native ligand with cyclocurcumin as the lowest score (-128.38 kcal/mol). This anti-viral activity was hypothesized from the similarity of hydrogen bonds with amino acid residues Ser 128 and Asn 40 as key residues present in Ribavirin. This study reveals that *C. longa* is the potential to be developed as an antiviral agent through replication inhibition in SARS-CoV-2 targeting its replication mediated by NSP3.

Keywords: *C. longa, Non-Structural Protein 3, COVID-19.*

INTRODUCTION

The World Health Organization (WHO) on March 11, 2020, declared the novel coronavirus disease 2019 (COVID-19) outbreak a global pandemic (Cucinotta and Vanelli, 2020). SARS-CoV-2 has a higher reproductive rate (R0) than SARS-CoV indicating this virus could spread much faster (Cevik, *et al*., 2020). The R0 of SARS-CoV-2 was estimated at 2.5 compared to 2.0–3.0 for SARS-CoV and the 1918 influenza pandemic, 0.9 for MERS-CoV, and 1.5 for the 2009 influenza pandemic(Petersen, *et al*., 2020). In severe cases, the sufferer will experience acute respiratory distress syndrome (ARDS), sepsis, multi-organ failure, and even death due to excessive cytokine production in the body (cytokine storm) (Tang, *et al*., 2020).

NSP3 encodes PLpro which is required for efficient cleavage of viral polyproteins, a process

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essential for transcription and replication of the viral genome (Albini, *et al.*, 2020). Considering the important activity in viral replication, inhibition of NSP3 activity is a potential target for antiviral activity. *C. longa* or turmeric has been documented to have a broad spectrum of pharmacological effects including antiviral, antifungal, anti-inflammatory, antioxidant, anticancer, antimicrobial, anticoagulant, and antidiabetic (Mitsuwan, *et al*., 2020). *C. longa* contains several active compounds curcumin, demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin which have pharmacophore 1,5-diaryl-3-oxo-1,4-pentadienyl, align at the auxiliary site and affect their biological activity (Chen, *et al*., 2016).

To understand the pharmacological and safety effects of *C. longa* as a potential therapy against SARS-CoV2, a bioactivity test is needed (Wolfram and Trifan, 2018). This test can be carried out through *in silico* studies with molecular docking techniques to demonstrate the binding affinity of compounds in *C. longa* to receptors on the SARS-CoV2 virus (Santos, *et al*., 2019). The advantages of this method include reducing the use of excessive tools and materials, faster time, and can save experimental costs (Dona, *et al*., 2019). The *in silico* method also emphasizes biological approaches that ensure quality and safety and makes recommendations for predicting biosafety on human exposure (Rim, 2020). Based on the urgency of SARS-CoV-2 and the need for therapy by focusing on the main target of viral replication activity and considering the potential compounds in *C. longa*, this study aims to find the binding interaction of Curcuminoid derivatives with SARS-CoV-2 targets through insilico studies.

METHODS

Ligand and Protein Preparation

Compounds of *C. longa*, the ligand employed in this paper, were downloaded as a SMILE file from PubChem (http://pubchem.ncbi.nlm.nih.gov/). The protein data bank website (https://www.rcsb.org/search) provided the protein NSP3 with PDB ID: 7KG3. Protein stabilization was carried out using Avogadro software adjusted human physiology by eliminating water and hydrogen atoms. The native ligand for 7KG3 was MES_203A.

Drug-likeness and Biological Activity Prediction

Using the SwissADME webserver (http://swissadme.ch), the *C. longa* ligand molecule was pharmacokinetically assessed as a drug candidate. The physicochemistry analysis was performed using the pkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction) and Protox (https://tox-new.charite.de/protox_II/). Additionally, toxicity prediction is used to assess the toxicity which includes lethal dose 50 (LD_{50}), skin classification, ames toxicity, hepatotoxicity, gastrointestinal absorption (GIA), the ability to penetrate blood-brain barrier (BBB), and skin permeability (SP) obtained from the web free of charge.

Molecular Docking

The binding energy value created when a ligand binds with its receptor is calculated using molecular docking. Comparing the binding energies of Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, and Cyclocurcumin compounds in *C. longa* and a native ligand that is attached to the same binding site in this study is known as "specific docking." In this work, molecular docking simulations are carried out using the Molegro Virtual Docker 6.0 software.

Protein-ligand Interaction Analysis

The molecular docking visualization was done using Molegro Virtual Docker 6.0 software. A study of protein-ligand bonding was conducted based on the interaction and kind of bond created by the *C. longa* compound when it binds to target proteins. The rerank score evaluated as Bond energy

Table 1. Pharmacokinetic test.

Active Ingredients	GIA (%)	BBB (log BB)	SP (log Kp)
Curcumin	75.275	0.571	-3.018
Demethoxycurcumin	92.740	0.346	-3.099
Bisdemethoxycurcumin	92.047	0.140	-3.077
Cyclocurcumin	91.950	0.461	-3.227
Ribavirin	54.988	0.921	-2.763

indicates the amount of energy required to form a bond between the ligand and the receptor. After docking with the earlier Molegro Virtual Docker 6.0 software, the software presented a realistic 3D schematic depiction of the intricate interaction between the ligand and the receptor.

RESULTS

Drug-likeness and the Biological Activity Prediction of *C. longa*

The pharmacokinetic tests in this study were carried out by uploading SMILES of the Active ingredients of herbal compounds on the SwissADME online site (http://swissadme.ch/). The analysis results showed the gastrointestinal absorption (GIA), the ability to penetrate blood-brain barrier (BBB), and skin permeability (SP) displayed in Table 1.

The Lipinski Rule of Five is used to determine the level of compound similarity that has a certain biological activity which can then be determined the compound feasibility as a candidate for a new drug. This test is carried out on the Lipinski test site pkCSM and Protox. The result showed in Table 2.

Molecular Docking

Molegro Virtual Docker software was used to conduct the molecular docking test. This assay was run to determine the molecular interaction of Active Ingredients of Compounds in *C. longa* as ligand with NSP3 as receptor compared with their native ligand to find binding affinity value through the rerank score. Table 4 displays the results of the binding affinity analysis.

Protein-ligand Interaction Analysis

Residue of amino acid can be observed from interaction analysis between ligand and protein target. The amino acid residue, distance, and ligan group in hydrogen interaction and steric interaction are presented in Table 5.

The result of the interaction on the docking native ligand, MES_203A is displayed in the best position in 3D are presented in Figure 1.

Table 2. Lipinski rule of five test results.

Active Ingredients	Molecular weight (g/mol)	Hydrogen bond donor	Acceptor bond donor	Log*P*	Rotatable bonds	Lipinski Rule Violation
Curcumin	368.38	2	6	3.03	8	0
Demethoxycurcumin	338.35	2	5	3.00	7	0
Bisdemethoxycurcumin	308.33	2	4	2.83	6	0
Cyclocurcumin	368.38	2	6	2.82	5	0
Ribavirin	244.20	1	2	2.18	3	0

Table 3. Toxicity test results.

Active Ingredients	Toxicity				
	Toxicity Class	LD_{50} (mg/kg)	Hepatotoxic	Skin Sensitivity	Ames Mutagenic Test
Curcumin	4	2000	No	No	No
Demethoxycurcumin	4	2000	No	No	No
Bisdemethoxycurcumin	5	2560	No	No	No
Cyclocurcumin	4	1500	No	No	No
Ribavirin	5	2700	No	No	No

DISCUSSION

This study showed that curcumin, demethoxycurcumin, bisdemethoxycurcumin, and cyclocurcumin have no Lipinski rule violation. An ideal drug molecule would comply with the physicochemical property guidelines of Lipinski's Rule of Five (RO5). This rule predicts the drug-likeness of a chemical compound with a certain biological activity designed for the oral route of administration(Chen, *et al*., 2020). Toxicity data is made available through the Globally Harmonized System of Classification and Labelling (GHS). Acute toxicity is the term used to describe the negative consequences that accompany the oral or topical administration of a single dose, repeated doses within a 24 h, or a 4 h inhalation exposure (United Nations, 2011). Acute toxicity values are expressed as (approximate) LD_{50} (oral, dermal) or LC_{50} (inhalation) values or as acute toxicity estimates (ATE). There are five acute toxicity categories defined by the GHS, ranging between category 1 (most severe) to category 5 (least severe) (Allen, *et al.*, 2019). The acute lethality hazard is typically determined using the LD_{50} which is defined as the median dose predicted to kill 50% of a given test population (Morris-Schaffer and McCoy, 2021). LD_{50} use to predict the category of GHS with a range of category 1 (≤5 mg/kg), category 2 (>5≤50 mg/kg), category (3>50≤300 mg/kg), category 4 (>300≤2000 mg/kg), category 5 (>2000 mg/kg) and Not Classified >5000 mg/kg. Cyclocurcumin with LD_{50} of 1500 mg/kg make its included in category 4, while other compounds are included in category 5 (Hamm, *et al*., 2021).

The visualization of molecular docking results in Figure 1 demonstrates how the biological activity of a protein may be affected by its contact with a ligand via the lowest energy bond. The smaller bond energy (Rerank Score) means a more stable bond. If the ligand binding to the receptor is more stable, it can be stated that the higher binding affinity and its activity are getting bigger (Kesuma, *et al.*, 2018) The difference in value is predicted because there are differences in the binding of the ligand to the amino acid at the NSP3 receptor so that the conformation can determine the most stable molecular geometry state. An increase in the hydrophilic nature of the resulting amino acid residues will lead to a lower value of the binding free energy (Shafhan, *et al*., 2020). The low binding energy shows that *C. longa* could be a substrate that stimulate biological activity and became an antiviral agent to limit coronavirus infection mainly

Table 4. Binding affinity result.

Reseptor	Rerank Score (Kcal/mol)					
	Curcumin	Demethoxy curcumin	Bisdemethoxy curcumin	Cyclo curcumin	MES_203A	Ribavirin
NSP3	-114.270	-111.967	-106.879	-128.38	-70.275	-127.920

Table 5. Active site, dimension, and docking center grid.

Compounds	Hidrogen interaction			Sterik interaction		
	Amino Acid	Distance (Å)	Ligan group	Amino Acid	Distance (Å)	Ligan group
Curcumin	Leu 126	2.62	O, 26	Ser 128	3.04	C, 22
	Ala 129	3.15	O, 26	Tyr 42	2.87	O, 15
	Asn 40	3.00	O, 7	Gly 47		O, 8
Demethoxy curcumin	Asp 22	2.76	O, 24	Ile 23	2.62	C, 20
	Leu 126	2.68	O, 7	Ala 154	3.17	C, 0
Bisdemethoxy curcumin	Asp 22	2.76	O, 22	Ile 23	2.84	C, 20
	Ala 38	3.13	O, 15	Leu 126	3.19	C, 3
Cyclo curcumin	Ser 128	3.35	O, 15	Phe 132	3.16	O, 15
	Leu 126	2.64	C, 2	Ala 154	2.99	C, 23
	Ile 23	3.04	O, 26	Asp 22	2.94	O, 26
Ribavirin	Ala 39	2.55	N, 15	Gly 97	3.09	O, 16
		2.86	O, 8		2.97	N, 15
	Val 95	3.22	N, 15	Gly 47	2.95	O, 6
	Ser 128	2.59	N, 12	Asn 40		O, 8
	Ala 38	3.10	O, 8			
	Val 49	3.29	O, 5			
MES_203A	Gly 130	2.86	O, 10	Ile 131		O, 10
	Ser 128	3.04	O, 11			
	Phe 132	3.12	O, 10			
	Asn 40	2.86	O, 0			

transcriptional replication. NSP3 detaches NSP1, NSP2, and itself from polyproteins and interacts with other viral NSPs and ribonucleic acid (RNA) to form a transcription-replication complex. Furthermore, NSP3 can interact with host proteins such as a protein-coding gene ring finger and Chy zinc finger domain containing 1 (RCHY1) to support viral survival (Forni, *et al*., 2017).

The presence of these same amino acid residues indicates that *C. Longa* has similarities with the native ligand in binding up with 7KG3 so that it can produce a similar effect as binding to the original ligand. The presence of hydrogen bonds produced during the bonding process and the high similarity of amino acid residues compared to the control are predicted to have a strong bond (Shafhan, *et al*., 2020). The closeness of the results between the test ligands and ribavirin suggests similar activity. Several studies have shown that ribavirin is useful for treating coronavirus infection because of its broad-spectrum inhibition of RNA viruses as SARS-CoV tested *in vitro* (Tong, *et al*., 2020).

Cyclocurcumin presents an almost optimal molecular structure with the presence of only one isomerize double bond, despite a non-negligible overlap between the absorption spectra of the two isomers. The presence of a single isomerize bond is highly desirable since it allows fine control of the photo switching activity between two defined states (*i.e.* trans and cis isomers, avoiding the complexity due to a higher number of states) (Marazzi, *et al*., 2020). The pharmacological effect of curcumin is due to the presence of phenolic and carbonyl hydroxyl groups that form hydrogen bonds of target molecules that affect the biological systems of organisms such as protein expression (Syarif, *et al*., 2016). Demethoxycurcumin consists of two aryl butene-2-one (feruloyl) chromophore coupled with a methylene group (Rothwell, *et al*., 2013). This chromophore has conjugated double bonds which can increase stability and decrease molecular energy (Suhartati, 2017).

Most individuals (90%) infected with SARS-CoV-2, an RNA virus that infects the

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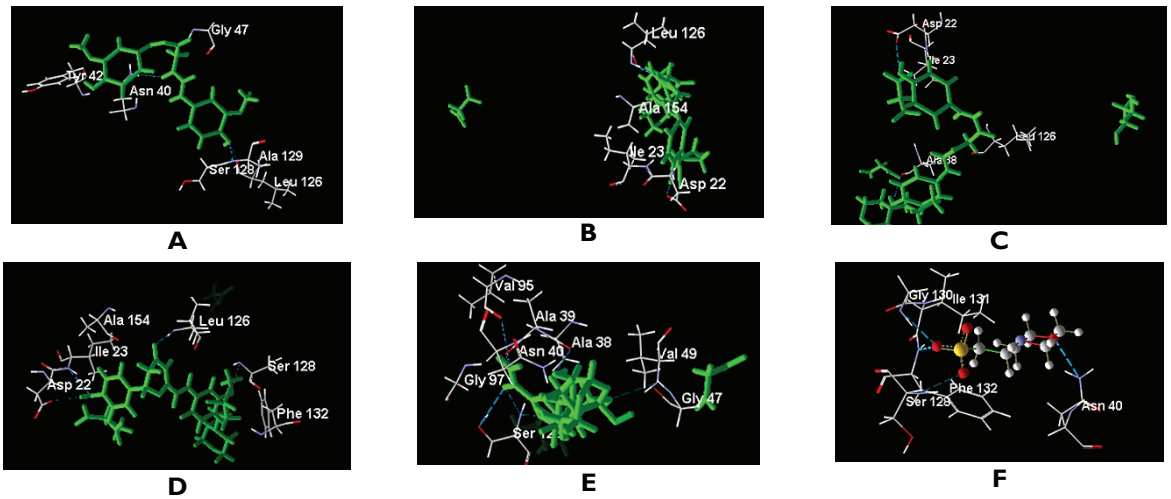

Figure 1. Visualization of 3D molecular interaction between NSP3 as receptor and Curcumin (A), Demethoxycurcumin (B), Bisdemethoxycurcumin (C), Cyclocurcumin (D), Ribavirin (E), dan native ligand MES_203A (F). Amino acid residues are displayed in stick mode and ligand poses with a wireframe model using color by the chain.

respiratory system, remain asymptomatic or develop a benign, self-healing, ambulatory illness of the respiratory tract (Casanova and Abel, 2021). Excessive tumor necrosis factor (TNF) production during some respiratory viral infections is associated with lung pathology and death. TNF is known to contribute to an exaggerated immune response leading to host tissue destruction and immunopathology during some viral infections (Junaliah, *et al.,* 2020). Cyclocurcumin inhibits the release of TNF-α as a key factor in a variety of inflammatory diseases in human macrophages. Study results demonstrated that treatment with cyclocurcumin at concentrations such as 10, 20, and 40 µM exhibited the least cytotoxic effects by evaluating p38α as regulator TNF-α expression. Thus, these data confirm the role of cyclocurcumin in overcoming p38α-induced production of TNF-α and hence can be used as a therapeutic agent to target inflammatory disease (Fu, *et al.,* 2017).

The study suggests that curcumin plays a role in preventing COVID-19 infection by inhibiting pathogen entry, viral genome replication, and steps in the endosomal pathway along with inhibition of T-cell signaling by impairing autophagy-mediated antigen-presenting pathways. *In silico* simulation suggests the binding efficiency of curcumin with the NSP3 may affect the RNA-dependent RNA polymerase (RdRp) activity of viral genome replication and double-membrane vesicle (DMV) formation (Dhar and Bhattacharjee, 2021). Cyclocurcumin, Bisdemethoxycurcumin, and Demethoxycurcumin were found to exhibit high affinity with SARS-CoV-2 ADP ribose phosphatase, NSP3 (Maurya and Sharma, 2020). The binding of these phytochemicals with NSP3 may slow down the cleavage of PPs to release NSPs and decrease the process of viral replication and transcription (Kim, *et al*., 2021). the predicted binding energy of different phytochemicals with molecules involved in the inflammatory processes such as COX2, PLA2, NIK, and IRAK-4 show that Cyclocurcumin may possess a high binding affinity with most of the inflammatory molecules (Maurya and Sharma, 2020).

This study is limited by its single protein target, other new proteins should be evaluated. However, such an approach is generally accepted in emerging diseases such as SARS-CoV-2 infection. 7KG3 as the model of NSP3, which only has

an MES buffer in the crystal structure is thought to affect the ability and strength of bonds with compounds. Further research can be developed using other receptor codes and other crystal structures which are complex with inhibitors.

The work described here outlines the means to produce an antiviral for SARS-CoV-2 proteins from the natural compounds and their use in the discovery of potential inhibitors, as demonstrated for 7KG3 as a proof of concept. These hits form the basis for medicinal chemistry development around the structural templates in search of antivirals conceived expressly for SARS-CoV-2. The field is open for such discovery efforts for most of these viral targets.

CONCLUSION

Curcumin, Demethoxycurcumin, Bisdemethoxycurcumin, and Cyclocurcuma compounds in *C. longa* could be an antiviral effect by limiting the replication processes of SARS-CoV-2 by binding up with NSP3 observed through the binding affinity was higher than its ligand and similar amino acid residue between the compounds and native ligand.

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