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Original Article

The Potential Effect of Nigericin from *Streptomyces hygroscopicus* subsp. Hygroscopicus Against the Syndemic of Malaria and COVID-19 through Molecular Docking Perspective

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ABSTRACT

Background: Malaria is a constantly challenging problem, notably in the Coronavirus Disease-19 (COVID-19) pandemic. The syndemic condition, malaria-COVID-19 co-infections, had been reported. Our previous study successfully revealed several compounds from *Streptomyces hygroscopicus* subsp. Hygroscopicus, including nigericin that has both antimalarial and antiviral effects. In malaria infection, *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) is the potential target for eliminating *Plasmodium*. Meanwhile, for SARS-CoV-2 infection, MPro is an essential protein for SARS-CoV-2 survival. This research aims to examine the potential effect of nigericin towards *Plasmodium* and SARS-CoV-2 by assessing its molecular interaction with PfCRT and MPro through molecular docking study. **Methods:** The protein target PfCRT and MPro were obtained from Protein Data Bank. Nigericin and the control ligand (chloroquine and N3) were obtained from PubChem. The pharmacokinetic analysis was done using SwissADME. Specific molecular docking was conducted using PyRx 0.9 and was visualized using LigPlot and PyMOL. **Results:** Nigericin has a large molecular weight, leading to the non-fulfillment of the Lipinski rule for oral administration. Through molecular docking study, the binding affinity of the Nigericin-PfCRT complex was -8.1 kcal/mol, and Nigericin-MPro was -8.6 kcal/mol. These binding affinities were stronger than the control ligand. The interaction between Nigericin-PfCRT and Nigericin-MPro share a similar pocket-site and amino acid residues as the control ligands. **Conclusion:** Nigericin has potential antimalarial and anti-coronavirus effects through molecular docking perspective by assessing the binding affinity and similarity of amino acid residues compared to control. Administration of systemic route can be an option in giving nigericin.

Key words: COVID-19, Malaria, Molecular docking, Nigericin.

INTRODUCTION

Malaria is a constantly challenging problem, notably in the Coronavirus Disease-19 (COVID-19) pandemic era. The latest malaria report revealed the 20 years of global progress and challenges influenced by multidimensional issues. There were 229 million new malaria infections in 2019, with 409 thousand people dying because of malaria in the same year. The number of malaria cases and deaths in 2019 had decreased compared with the report in 2000, 238 million and 736 thousand, respectively. Although the global trends in the burden of malaria were reported in a declined course, malaria elimination and prevention are one of the global concerns, especially in the development of vaccines and novel antimalarials due to its resistance.[1]

COVID-19 was confirmed as an outbreak in Wuhan City, China, caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) at the end of 2019. The emergence of COVID-19 generates the novel viral pneumonia spreading to other cities in China and the neighboring countries. The virus aggressively spread across the continents until it was declared by World Health Organization (WHO) to be a pandemic in March 2020.[2,3] The highly contagious virus spread to all malaria-endemic countries, including the South-East Asia Region, by April 2020.[3,4]

Recently, the case of Malaria-COVID-19 co-infections had been reported. The clinical presentation of the patient with this co-infection was varied. Malaria and COVID-19 share a similar manifestation, known as the pro-coagulation state.[2] Both infections that coincide may interact and result in severe forms of the disease called syndemic conditions. The diagnosis is difficult to establish a single or co-infection state because of the similar incubation period between malaria and COVID-19.[5,6] Facing both diagnoses is challenging, particularly for physicians in malaria-endemic countries, including Indonesia.

Malaria and COVID-19 syndemics have been considered to discover a new drug that has a dual effect on the parasite and the virus. Previous antimalarial drugs such as chloroquine and hydroxychloroquine had been used to treat COVID-19. Although WHO had no longer recommends these drugs,[7] it is hypothesized that the use of chloroquine and hydroxychloroquine is associated with a low prevalence of COVID-19 in malaria-endemic countries.[2] These findings provide the possibility of using chloroquine and other potential antimalarial drugs to treat COVID-19 and malaria.

Our previous study found several compounds from *Streptomyces hygroscopicus* subsp. Hygroscopicus has antimalarial and antivirus effects. One of the

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finding compounds from the bacteria is nigericin. Nigericin is a polyether ionophore which widely used in Coccidiosis.[8] This compound is believed to have a mechanism of action similar to chloroquine.[9] By exchanging cation transport to the cell membrane, nigericin can increase alkalinization within the food vacuole and inhibit protein degradation in the lysosome.[10]

One of the potential therapeutic targets in malaria infection is *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT). PfCRT is a molecule transporter that plays a role in drug and nutrient exporters, crucial for parasite survival.[11,12] Meanwhile, for SARS-CoV-2 infection, due to its new variant coronavirus, the research to find the appropriate therapeutic intervention is still ongoing. However, MPro SARS-COV-2 has been known as a therapeutic target for anti-coronavirus drug development.[13] MPro is believed to be an essential survival component of the virus due to the responsibility for the replication and transcription process for SARS-CoV-2 proteins.[14]

This research aims to examine the potential effect of nigericin on *Plasmodium* and SARS-CoV-2 by assessing its molecular interaction with PfCRT and MPro SARS-CoV-2 through a molecular docking perspective.

MATERIALS AND METHODS

Design and settings

The exploratory method study design was applied using Liquid Chromatography/Mass Spectrometry (LC/MS); then, the in silico approach was done by assessing protein-ligand interactions in specific molecular docking to reveal the antimalarial activities of the identified compound. *Streptomyces hygroscopicus* subsp. Hygroscopicus culture was carried out in the Laboratory of Microbiology, Faculty of Medicine, UniversitasBrawijaya. The extraction process was conducted at the Laboratory of Pharmacy, Faculty of Medicine, UniversitasBrawijaya. LC/MS analysis was done at the Inbio Indonesia, and the *in silico* study was carried out at the Laboratory of Parasitology, Faculty of Medicine, Universitas Brawijaya.

Culture of *Streptomyces hygroscopicus* subsp. Hygroscopicus

Streptomyces hygroscopicus subsp. Hygroscopicuswas was obtained from LIPI Microbial Collection, Cibinong, Indonesia. The confirmation of the *Streptomyces hygroscopicus* subsp. Hygroscopicus colony was done by identifying the macroscopic morphology and microscopic Gram staining. The bacteria are Gram-positive with endospore, and it generates hyphae.

Secondary metabolite fermentation of *Streptomyces hygroscopicus* subsp. Hygroscopicus

The fermentation process was conducted in 50 ml of ISP4 broth media under aseptic conditions in 250 Erlenmeyer flask. The colony of bacteria was scratched from medium culture and homogenized in ISP4 broth media. Then, it was incubated at 28°C on a 150 rate per minute shaking incubator for 5 days. The inoculum for the process of fermentation was ready to use. The adjusted pH (7.0-7.4) of 100 mL ISP4 broth was autoclaved, then 25.8 x 106 bacteria from inoculum were added to the broth and fermented at 28°C with 150 rpm of shaking incubator for 5 days.[15,16]

The extraction process for metabolites of *Streptomyces hygroscopicus* subsp. Hygroscopicus

After 5 days of fermentation, the medium was centrifuged to remove cells and cell debris to produce filtrate and fermentation broth. The filtrate was collected in a separating funnel, and was mixed with 1:1 (v/v) ethyl acetate. and hand-shaken for 1 hour. The solvent phase containing metabolic compounds was separated from the aqueous phase and was evaporated to dryness in a water bath.

Secondary metabolite extract fractionation of *Streptomyces hygroscopicus*

The fraction was generated on a BUCHI Reveleris® PREP Purification System flash column with silica gel 60 which was eluted in a 1:1 (v/v) gradient mode using n-hexane and ethyl acetate. UV light detector of 254nm, 365nm, 366nm and Evaporative Light Scattering Detector (ELSD) were applied. The flow rate used was 5mL / minute. The result of fractionation were collected in vial and were evaporated to remove the solvent.

Liquid chromatography/mass spectrometry (LC/MS) analysis

Fractions from secondary metabolite extract samples of *Streptomyces hygroscopicus* subsp. Hygroscopicus were injected and analyzed using the Ultra-Performance Liquid Chromatography (UPLC) and ultra-high-resolution time-of-flight mass spectrometry (TOF-MS) detector.

Pharmacokinetic profile

The identified metabolite, i.e., nigericin, from *Streptomyces hygroscopicus* subsp. Hygroscopicus was analyzed for the pharmacokinetic profile using SwissADME (http://www.swissadme.ch/) by entering the SMILES formula.

Ligand preparation

The 2D structure of Nigericin from *Streptomyces hygroscopicus* subsp. Hygroscopicus was downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and saved in the .sdf extension. The 2D structure was converted into a 3D structure and minimized the energy using OpenBabel. The control ligands in this study were chloroquine to assess the antimalarial potential and N3 to assess the antiviral potential of SARS-CoV-2.[17]

Receptor preparation

The target proteins PfCRT (PDB ID: 6UKJ) and MPro SARS-CoV-2 (PDB ID: 6LU7) were downloaded from the Protein Data Bank (www.rscb.org) and then optimized by removing existing residues such as water, co-crystallized ligands, and co-factors using PyMOL. The structure is saved in the .pdb extension.

Specific docking

Nigericin and control ligands were carried out by specific molecular docking. The active site of PfCRT protein target lies on amino acids 59 to 79 (Antony *et al.*, 2019). The pocket site from MPro SARS-CoV-2 coordinated at X = -12.71, Y = 17.14, and Z = 65, 92 with dimensions of 30x30x30 A.[18]

Interaction visualization

The molecular docking results are visualized for the residue interactions, both two-dimensional and three-dimensional, using LigPlot and PyMOL.

RESULTS

LC/MS identification

The result of the LC/MS showed that the fraction from secondary metabolite extract samples of *Streptomyces hygroscopicus* subsp. Hygroscopicus contained abundant active compounds from polyether ionophore groups, such as nigericin, lenoremycin, dianemycin, carriomycin, etheromycin, and septamycin. From those six compounds,

nigericin has the highest peak which shows a highest ion abundance as shown in Figure 1. The identified compound from fraction of *Streptomyces hygroscopicus* subsp. Hygroscopicus is shown in Table 1.

Pharmacokinetic prediction of nigericin

Predictive analysis of the pharmacokinetic profile of the active compound was conducted in the form of analysis of the absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile of the active compound. Nigericin, as shown in Figure 2, has a molecular weight of 724.96 g/mol. The hydrogen bond donor and acceptor of nigericin are 3 and 11, respectively. The lipophilicity properties of nigericin from the cLogP value is 4.57. Veber's rule criteria components consisting of rotatable bond and TPSA value of nigericin are 9 and 142.37 Å^2. The pharmacokinetic prediction of nigericin is shown in Table 2.

Molecular docking

The result of molecular docking is the binding affinity between the target protein and the active compound. The binding affinity of PfCRT-Nigericin and MPRO-Nigericin was compared with the binding of the two protein targets with each control ligand to determine the prediction of the binding strength of each compound to the target protein. The binding affinity of PfCRT-Nigericin was -8.1 kcal/mol compared to the control (chloroquine) of -6.37 kcal/mol. The binding affinity of MPRO-Nigericin was -8.5 kcal/mol compared to the control of -7.8 kcal/mol. The binding affinity results of Nigericin with PfCRT and MPRO compared to control are shown in Figure 3.

Interaction visualization

The active compound nigericin bound to the target protein PfCRT through hydrophobic interactions with Ile358, Phe382, Ile351, Leu381, Tyr384, Tyr62, Val348, Ser388, Thr344, Phe378, Ile347, Ile389, Leu385, Leu69, but did not form hydrogen bonds like control ligands. The active compound nigericin bound to the target protein MPro through hydrophobic binding to Thr25, Thr26, Leu27, Met49, Asn142, Cys145, His163, His164, Met165, Glu166, Pro168, Asp187, Arg188, Gln189, Thr190. In contrast to PfCRT-Nigericin, which did not form hydrogen bonds, MPro-Nigericin formed hydrogen bonds with His41 and Gly143. The binding interactions of nigericin with PfCRT and MPro are shown in Table 3. The visualization of the interactions of nigericin with PfCRT and pocket-site proteins is shown in Figure 4, while nigericin with MPro is shown in Figure 5.

DISCUSSION

Nigericin is one of the polyether ionophore groups. This compound is often used as an anticoccidial agent.[8,19] Bacteria, especially *Streptomyces hygroscopicus* (isolated from the soil), are the primary producers of nigericin.[20] Several studies have stated that this compound is useful as an anticoccidial, antibacterial, antifungal, antiparasitic, antiviral, cardiovascular agent, immunoregulatory, anti-inflammatory, herbicide, and has cytotoxic effects on tumor cells.[8,21] In addition, this compound can effectively kill cancer stem cells and multidrug-resistant cancer cells.[8]

Based on the Lipinski Rule of Five, nigericin did not meet the criteria for oral administration because of its large size (**Table 2**). Meanwhile, based on Veber's criteria, the TPSA of Nigericin compounds exceeds 140 A; thus, the ability to transport molecules through cell membranes is inadequate, especially during the absorption process.[22] However, the lipophilicity value of nigericin is small, which means it has good lipophilicity. Modifying the formulation of the compound or changing the route of administration can increase the absorption process in the body and increase the effectiveness of the compound as a therapeutic agent.

Nigericin has high antimalarial potential because it kills the malarial parasite by disturbing ion homeostasis at a relatively low effective dose.[23] Nigericin is known to act by entering the membrane of intracellular organelles and exchanging protons for cations, leading to alkalinization of the food vacuole and inhibition of lysosomal protein degradation.[9]

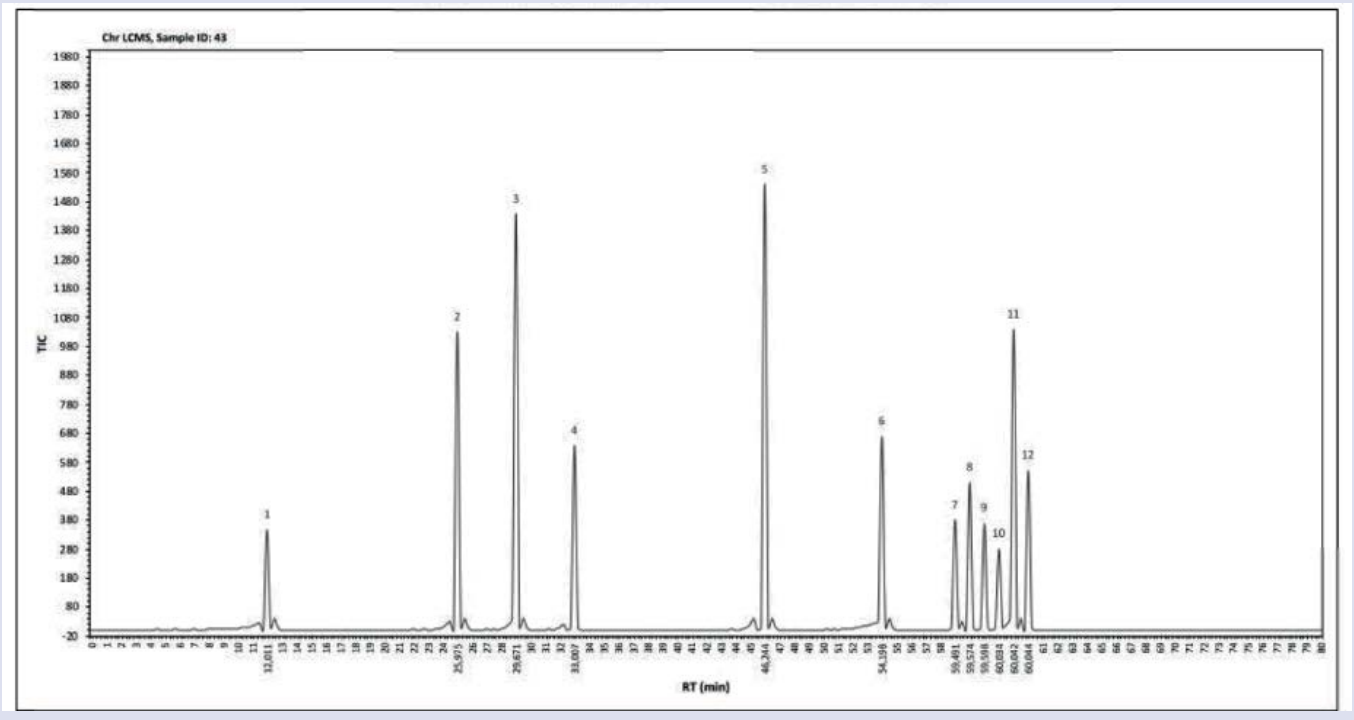

Figure 1: Chromatogram result of fraction from secondary metabolite extract samples of *Streptomyces hygroscopicus* subsp. Hygroscopicus.

Table 1: Identified compound from fraction of *Streptomyces hygroscopicus* subsp. Hygroscopicus through LC/MS.

No	RT	Similarity index (%)	Curve area	Composition (%)	Chemical formula	Possibility name	Exact mass
1	12.011	92	346.15727	3.94451	C11H21N3O6P	Bialaphos	322.1173
2	25.975	92	1028.60571	11.72110	C20H35NO13	Validamycin	497.2108
3	29.671	92	1435.92772	16.3259	C20H37N3O13	Hygromycin B	527.2326
4	33.007	92	637.37518	7.26298	C29H40N2O9	Geldanamycin	560.2734
5	46.244	92	1537.68566	17.52214	C40H68O11	Nigericin*	724.4762
6	54.198	92	667.28858	7.60385	C44H76O14	Emericid	828.5235
7	59.491	92	380.16762	4.33206	C47H78O13	Lenoremycin*	850.5442
8	59.574	92	509.39897	5.80467	C47H78O14	Dianemycin*	866.5392
9	59.598	92	367.02277	4.18228	C47H80O15	Carriomycin*	884.5497
10	60.034	92	279.37888	3.18356	C51H79NO13	Rapamycin	913.5551
11	60.042	92	1037.26875	11.81982	C48H82O16	Etheromycin*	914.5603
12	60.044	92	549.39532	6.26044	C48H82O16	Septamycin*	914.5603

Table 2: Pharmacokinetic prediction of nigericin.

Name	Molecular weight (g/mol)	H-bond donor	H-bond acceptor	cLogP	Rotatable bond	TPSA (Å^2)
Nigericin	724.96	3	11	4.57	9	142.37

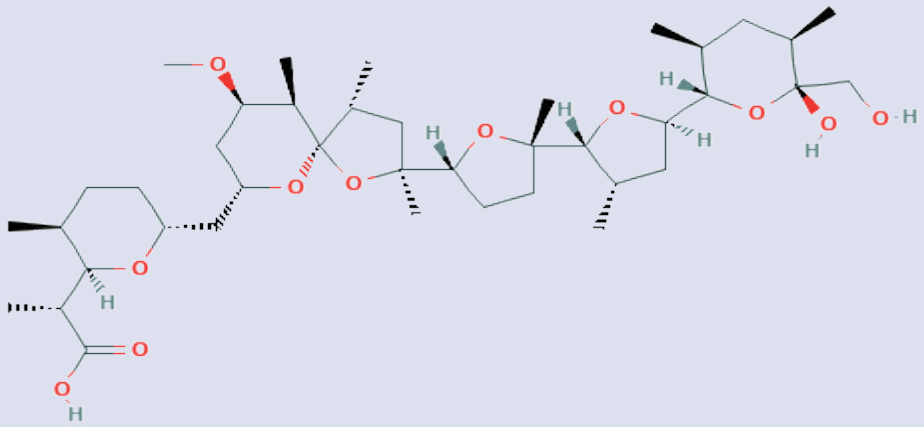

Figure 2: Chemical structure of nigericin.[17]

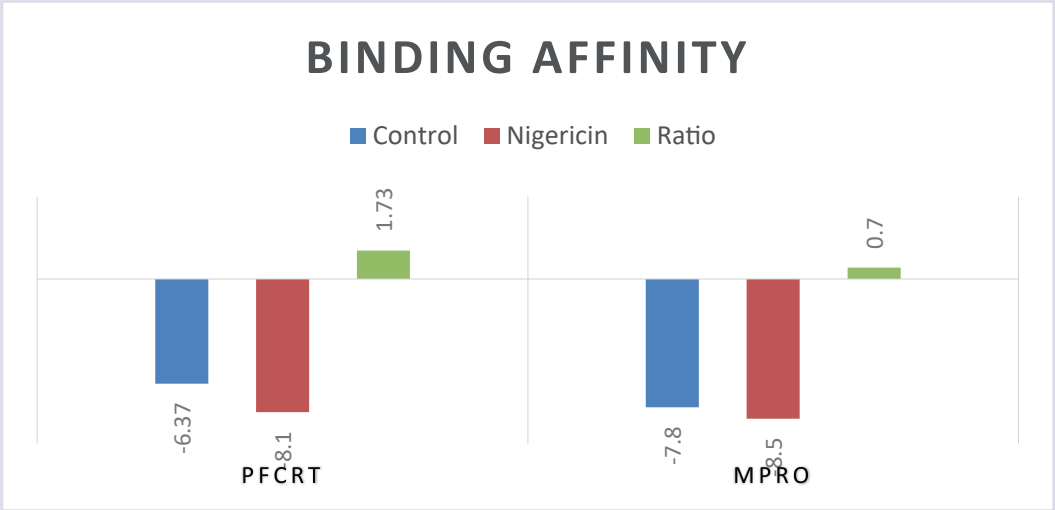

Figure 3: Binding affinity comparison between nigericin and control ligand complexes for PfCRT and MPro.

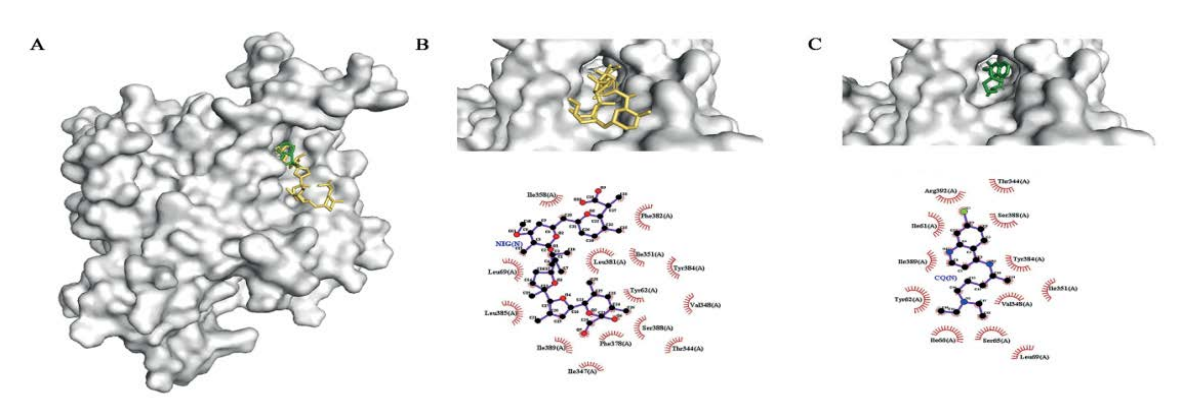

Figure 4: The visualization interaction between nigericin, chloroquine, and PfCRT (A). nigericin (yellow) shares the same pocket-site with chloroquine (green) formed complexes to PfCRT (white) (B, C).

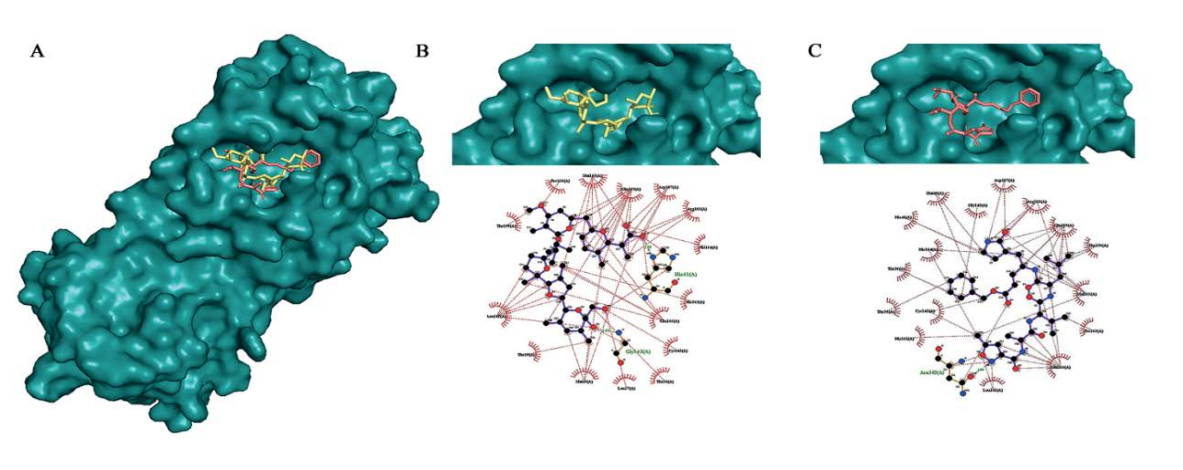

Figure 5: The visualization interaction between nigericin, N3, and MPro (A). nigericin (yellow) shares the same pocket-site with N3 (orange) formed complexes to MPro (green) (B, C).

Table 3: Interaction between nigericin-PfCRT and MPro compared to control.

PDB ID	Protein target	Ligand	Interaction with the protein target	The bond
6UKJ	PfCRT	Nigericin	Hydrogen bond: -	0
			Hydrophobic interaction: Ile358, Phe382**, Ile351**, Leu381, **Tyr384,Tyr62,Val348,Ser388, Thr344**, Phe378, Ile347, **Ile389**, Leu385, **Leu69**	14
		Control	Hydrogen bond: -	0
			Hydrophobic interaction: Arg392, **Thr344,Ser388,Tyr384**, **Ile351**, **Val348**, Ser65, **Leu69,** Ile66, **Tyr62,Ile389,** Ile61	12
6LU7	MPro	Nigericin	Hydrogen bond: His41, Gly143	2
			Hydrophobic interaction: **Thr25**, **Thr26**, Leu27, **Met49**, Asn142, **Cys145**, **His163**, **His164**, **Met165**, **Glu166**, **Pro168**, **Asp187**, **Arg188**, **Gln189**, **Thr190**	15
		Control	Hydrogen bond: Asn142	1
			Hydrophobic interaction: **Thr25**, **Thr26**, His41, **Met49**, Leu141, Gly143, **Cys145**, **His163**, **His164**, **Met165**, **Glu166**, **Pro168**, **Asp187**, **Arg188**, **Gln189**, **Thr190**	16

*__Bold__: The same interaction.

The protein *Plasmodium falciparum* Chloroquine Resistance Transporter (PfCRT) as a protein target for nigericin is part of the drug/metabolite transporter (DMT) superfamily that alters the acidic membrane of the digestive vacuole (DV) of parasites.[24–26] PfCRT is very important in parasite growth and replication and has been identified as one of the protein targets in developing antimalarial drugs. In the process of antimalarial drug discovery, PfCRT proteins can recognize various cation substrates, similar to polyspecific drug transporters, so that multi-pharmaceutical transporter inhibitors can be good candidates for PfCRT,[11] including nigericin. Inhibition of PfCRT can cause parasite death.[12]

The molecular docking results of nigericin showed more negative binding affinity than chloroquine as a control ligand (Figure 3). The results indicate that nigericin has a stronger binding ability when interacting with PfCRT. Similar to this result, it was reported that the antimalarial activity of nigericin at the nanomolar and picomolar scales was 30,000 times that of chloroquine.[9] By forming the same hydrophobic interactions with chloroquine (Figure 4, Table 3), Ile351, Tyr384, Tyr62, Val348, Ser388, Thr344, Ile389, Leu69, it indicates that nigericin can bind to the same active site as the control ligand, chloroquine.

Apart from being a potential antimalarial drug, nigericin has antiviral effects, both DNA and RNA viruses.[21] In vitro studies demonstrated the ability of nigericin against SARS-CoV-2 infection with IC50 of 11.25 μM.[27,28]

The MPro or 3CL Protease as the protein target for nigericin is essential for SARS-CoV-2 survival.[29,30] The primary function of MPro is to release functional polypeptides from polyproteins through proteolytic processes in replication and transcription.[29,30] MPro is also used by coronavirus to interact with the Angiotensin-Converting Enzyme 2 (ACE-2) receptor to enter the host cell. Thus, inhibition of this enzyme can disrupt the replication and transcription of viral proteins and halt the entry of the virus into cells, thereby preventing further infection.[14,28,29]

Nigericin could bind to MPro with -8.5 kcal/mol energy based on the binding affinity. This energy is lower than the control ligand N3, which only binds at -7.8 kcal/mol. A study by Jin *et al*.[13] revealed that the control ligand, N3, exerted the most substantial irreversible inhibitory effect on MPro by binding to the protein pocket-site through hydrogen bonds, thereby inactivating the protein.

The minimal energy from the Nigericin-MPro interaction indicates that nigericin requires less energy to form a bond with MPro, which shows that it is stronger than N3. In addition, the similarity of residues between the Nigericin-MPro complex compared to N3-MPro complex (Figure 5, Table 3), namely Thr25, Thr26, Met49, Cys145, His163, His164, Met165, Glu166, Pro168, Asp187, Arg188, Gln189, Thr190, also indicates that nigericin interacts at the same pocket-site as N3.

CONCLUSION

Nigericin can be antimalarial and antiviral for SARS-CoV-2 by assessing binding affinity and residue similarity compared to control ligands. The binding affinity of Nigericin to PfCRT and MPro is stronger than the control ligand. *In vitro* and *in vivo* studies are needed to further determine the therapeutic effect of nigericin.

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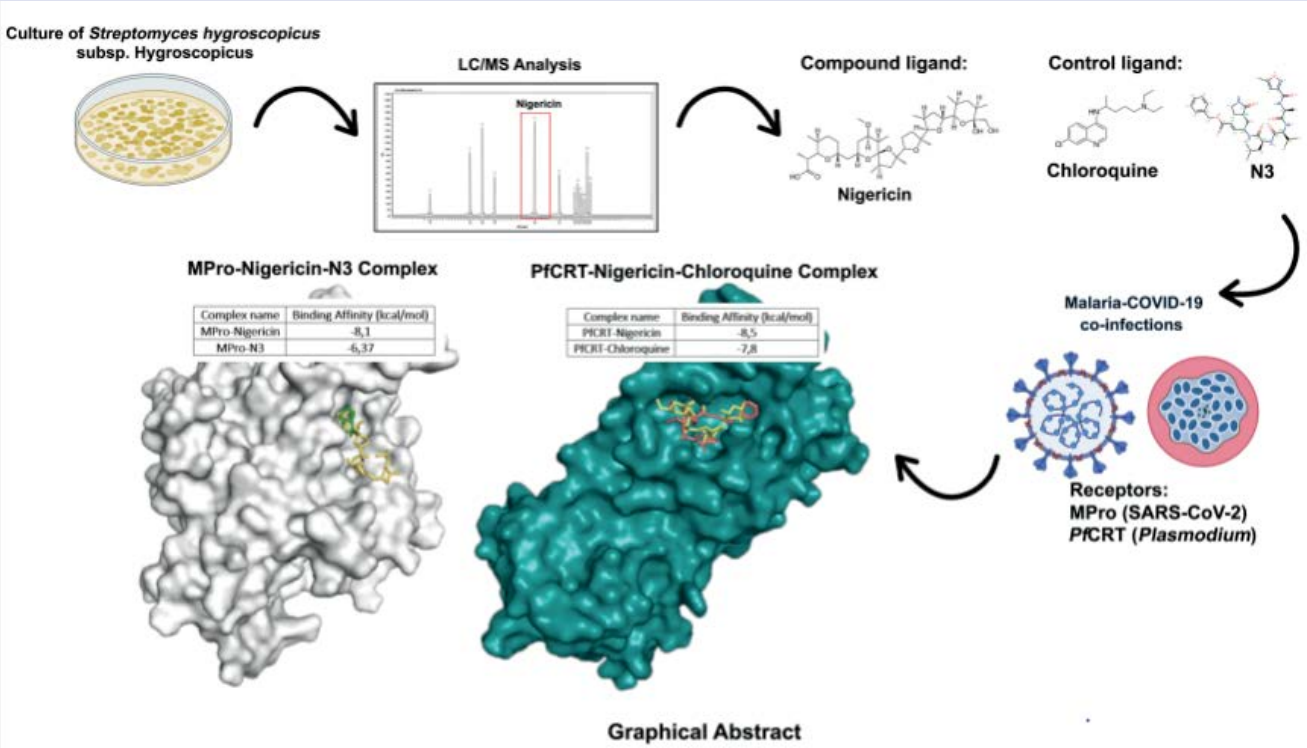
GRAPHICAL ABSTRACT



Graphical Abstract

Malaria and COVID-19 syndemics are being considered to discover a new drug that has a dual effect on the parasite and the virus. Nigericin is known to have antimalarial as well as antivirus activity. This compound was successfully detected from *Streptomyces hygroscopicus* Subspesies Hygroscopicus extract. Through the molecular docking approach, nigericin has a stronger binding affinity to *Plasmodium falciparum* chloroquine resistance transporter (*Pf*CRT) and M-Protein of SARS-CoV-2 (MPro) compared to chloroquine and N3, respectively. Nigericin also forms the same amino acid residues to the protein compared to those of the control. In conclusion, by assessing the binding affinity and residue similarity, nigericin can be a potential antimalarial and antiviral for SARS-CoV-2.

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