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Original Article

Antimalarial dihydrochalcone isolated from *Artocarpus sericicarpus* Jarret leaves and *in silico* investigation against falcipain-2 protein

[Dihidrochalcona antipalúdica aislada de las hojas de *Artocarpus sericicarpus* Jarret e investigación *in silico* contra la proteína falcipaina-2]

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Abstract

Aims: To investigate the potential antimalarial plant belonging to the *Artocarpus* genus, *Artocarpus sericicarpus*, and isolate the antimalarial active compound from leaves extract.

Methods: The isolation of the antimalarial compound was based on bioassay-guided isolation. The compound isolation was conducted by chromatography and identified using spectroscopic techniques, including ^{1}H, ^{13}C, and 2D NMR. The compound was tested for its antimalarial activity against *Plasmodium falciparum* and evaluated for cytotoxicity using several cell lines, including Huh7, HepG2, BHK-21, and Vero cells. *In silico* investigation of the compound against falcipain-2 protein was conducted as well.

Results: Fractionation and purification of dichloromethane leaves extract led to the isolation of a dihydrochalcone compound identified as 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-en-1-yl)phenyl]propan-1-one. The dihydrochalcone compound exhibited antimalarial activity with an IC_{50} value of 34.80 µM. Cytotoxicity test revealed CC_{50} values of the compound were more than 20 µg/mL, and the selectivity indexes (SI) were 4.50, 5.52, 3.02, and 6.68 on Huh7, HepG2, BHK-21, and Vero cells, respectively. The CC_{50} and SI indicated the nontoxic criteria of the compound. *In silico* investigation showed that the compound could bind to the falcipain-2 active sites.

Conclusions: The dihydrochalcone compound identified as 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-en-1-yl)phenyl]propan-1-one was isolated from *Artocarpus sericicarpus* leaves extract showed antimalarial activity and the ability to bind to the falcipain-2 active sites on *in silico* investigation.

Keywords: *Artocarpus sericicarpus*; dihydrochalcone; falcipain-2; malaria.

Resumen

Objetivos: Investigar la potencial planta antimalárica perteneciente al género *Artocarpus*, *Artocarpus sericicarpus*, y aislar el compuesto activo antimalárico del extracto de las hojas.

Métodos: El aislamiento del compuesto antimalárico se basó en el aislamiento guiado por bioensayo. El aislamiento del compuesto se realizó por cromatografía y se identificó mediante técnicas espectroscópicas, incluyendo ^{1}H, ^{13}C y 2D NMR. Se comprobó la actividad antimalárica del compuesto frente a *Plasmodium falciparum* y se evaluó su citotoxicidad en varias líneas celulares, como Huh7, HepG2, BHK-21 y Vero. También se llevó a cabo una investigación *in silico* del compuesto frente a la proteína falcipaína-2.

Resultados: El fraccionamiento y purificación del extracto diclorometano de las hojas condujo al aislamiento de un compuesto de dihidrochalcona identificado como 1-(2,4-dihidroxifenil)-3-[4-hidroxi-3-(3-metilbut-2-en-1-il)fenil]propan-1-ona. El compuesto dihidrochalcona mostró actividad antipalúdica con un valor IC_{50} de 34,80 µM. La prueba de citotoxicidad reveló valores CC_{50} del compuesto superiores a 20 µg/mL, y los índices de selectividad (SI) fueron de 4,50; 5,52; 3,02 y 6,68 en células Huh7, HepG2, BHK-21 y Vero, respectivamente. El CC50 y el SI indicaron los criterios de no toxicidad del compuesto. La investigación *in silico* demostró que el compuesto podía unirse a los sitios activos de la falcipaína-2.

Conclusiones: El compuesto dihidrochalcona identificado como 1-(2,4-dihidroxifenil)-3-[4-hidroxi-3-(3-metilbut-2-en-1-il)fenil]propan-1-ona fue aislado del extracto de las hojas de *Artocarpus sericicarpus* mostró actividad antimalárica y la capacidad de unirse a los sitios activos de falcipaina-2 en la investigación *in silico*.

Palabras Clave: *Artocarpus sericicarpus*; dihidrochalcona; falcipaina-2; malaria.

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INTRODUCTION

World Health Organization (WHO) reported there were 241 million cases of malaria worldwide in 2020 (WHO, 2021). Malaria eradication was burdened by *Plasmodium falciparum*'s resistance to the present antimalarial drugs. Chloroquine resistance was initially reported in 1957 in Thailand and spread to South Asia and South East Asia (Packard, 2014). There were several reports on the emergence of an artemisinin-resistant strain of *P. falciparum* in the Greater Mekong area (Fairhurst and Dondorp, 2016). Artemisinin combination therapy is the last defense against this dreadful parasite. There is an urgent need for antimalarial drug development.

The investigation of plants as antimalarials has contributed to antimalarial compounds discovery (Ginsburg and Deharo, 2011). Many antimalarial drugs today are derivatives of quinine and artemisinin. Both were first identified in the plants used traditionally to treat malaria and fever. Phytochemical investigation of the *Artocarpus* genus has found various flavonoids, terpenoids, stilbenoids, arylbenzofuran, neolignane and Diels-Alder adduct compounds (Hakim, 2010). The research in this laboratory on various *Artocarpus* plants, including *Artocarpus champeden*, *A. heterophyllus*, *A. altilis*, and *A. camansi* yielded mostly prenylated flavonoids with potent antiplasmodial activity (Boonphong et al., 2007; Hafid et al., 2012; 2016; Hidayati et al., 2020; Widyawaruyanti et al., 2007). A previous study in this laboratory reported a dihydrochalcone with strong antimalarial activity and possesses a cysteine protease inhibitor of falcipain-2 (Hidayati et al., 2020).

Falcipain-2 is a cysteine protease key enzyme involved in the life cycle of *P. falciparum* like hydrolysis of host hemoglobin, erythrocyte invasion, and erythrocyte rupture which constitute a new target in the search for new antimalarial drug and would solve the problem of current antimalarial drug resistance. Several phytochemicals have been identified as inhibitors of falcipain-2, such as isoquinolines, thiosemicarbazones, and chalcones (Ettari at al., 2010; Marco and Coterón, 2012). The chemotaxonomic relationship approach is one strategy in the search for new drugs from plants. Therefore, investigating antimalarial compounds from the *Artocarpus* genus and identifying the drug target as a falcipain-2 inhibitor is a prospective study to find new antimalarial substances.

Artocarpus sericicarpus Jarret leaves are investigated in the present study as part of research on *Artocarpus* plants as antimalarial. *Artocarpus* is the main genus of the *Moraceae* family, composed of 50 species, and 25 species of them exist on the island of Kalimantan. This genus has the potential to be a source of bioactive phytochemicals and is also used in traditional medicine (Kochummen, 1987; Verheji and Coronel, 1992). The study of antimalarial compounds from the *Artocarpus* genus will bring advantages in searching for new antimalarial from plants and provide a scientific explanation of using *Artocarpus* as traditional medicine. *Artocarpus sericicarpus* is locally known as peluntan or pedalai, and its growth is endemic to several areas in Indonesia, including Kalimantan, Sulawesi, and Maluku (Lim, 2012). The previous studies showed that the extracts and fractions of *A. sericicarpus* exhibited potential antimalarial activity (Tumewu et al., 2021).

There are many reports on *Artocarpus* plants, but little attention was put on *A. sericicarpus*. To the best of our knowledge, this is the first study on the isolation of antimalarial compounds from *A. sericicarpus* leaves. This study reports the isolation and structure elucidation of the antimalarial compound from *A. sericicarpus* leaves extract, its antimalarial activity, cytotoxicity, and *in silico* investigation against the falcipain-2 protein.

MATERIAL AND METHODS

General methods

The chemical shifts of compound **1** (δ_H and δ_C) in deuterated acetone ($(CD_3)_2CO$) were measured at 400 MHz NMR (^1H) and 100 MHz (^{13}C) using NMR instrument JEOL ECS-400. The m/z and chemical formula of compound **1** in acetone was determined by high-resolution mass spectrometry (MS) (Waters LCT Premier™ XE). The UV Shimadzu-1800 spectrophotometer measured the maximum absorption (λmax) of compound **1** in methanol. The Bruker Alpha II measured the absorption bands of the IR spectrum (cm^{-1}) of compound **1**.

Plant material

Leaves of *A. sericicarpus* were collected from the Balikpapan conservation forest, East Kalimantan, Indonesia, (116° 51′ 0.55″ - 116° 51′ 54.4″ East Longitude (EL) and LS 01° 07′ 49.2″ - 01° 08′ 46.6″ South Latitude (SL). Plant identification was performed by botanist Deden Mudiana, and the voucher specimen was deposited at the Indonesian Institute of Science, UPT Balai Konservasi Tumbuhan Kebun Raya Purwodadi, with the voucher number 0074/IPH.06/HM/XII/2015.

Extraction and isolation

Artocarpus sericicarpus leaves were dried at room temperature (25-28°C) with no direct sunlight exposure for approximately seven days. The dried leaves were further ground to obtain powdered leaves. The powdered leaves of *A. sericicarpus* (800 g) were extracted using n-hexane and dichloromethane sequentially by ultrasonic-assisted extraction for 3 × 2 min (equipment SONICA® 5200Ep S3, SOLTEC, Italy, 230/240 V ~50/60 Hz, 1400 W). The total solvent used was 4 L. The extracts were filtered and concentrated to afford n-hexane extract (3.77 g) and dichloromethane extract (16.07 g). The dichloromethane extract was subjected to polyamide open-column chromatography and eluted using n-hexane-ethyl acetate to afford two fractions (A and B). Fraction B (1.8 g) was further purified by silica open column chromatography eluted using gradient hexane-ethyl acetate (9:1 to 1:1 v/v) to afford six fractions (B1-B6). Fraction B5 (107.6 mg) was further purified using Sephadex LH-20 open-column chromatography eluted using methanol to afford two fractions (B5.1 and B5.2). Fraction B5.2 was further purified using radial planar chromatography on silica gel (hexane-acetone 95:5 to 85:15 v/v gradient) to afford three fractions (B5.2.1-B5.2.3). Fraction B5.2.3 (43.5 mg) was further purified using radial planar chromatography on silica gel (hexane-diisopropyl ether 1:1 to 3:7 v/v gradient) to yield compound **1** (6 mg).

In vitro culture of *Plasmodium falciparum*

The *Plasmodium falciparum* strain 3D7 was cultivated in human O+ red blood cells and complete RPMI-1640 medium (RPMI 1640 medium with L-glutamine and 25 mM HEPES) supplemented with 2 g/L sodium hydrogen bicarbonate ($NaHCO_3$), 50 mg/L hypoxanthines, 50 µg/mL gentamicin sulfate, and 0.5% (w/v) Albumax II under a standard gas mixture consisting of 90% N_2, 5% CO_2 and 5% O_2. The culture medium was repealed daily, and a Giemsa-stained thin blood smear monitored the parasitemia level (Trager and Jensen, 1976).

LDH antimalarial assay

One microliter of samples (concentration 50, 10, 5, 1, 0.5, 0.1, 0.05, and 0.01 µg/mL) and chloroquine diphosphate (concentration 5, 1, 0.5, 0.1, 0.05, 0.01, 0.005, and 0.001 µg/mL) as a standard drug were performed in 96-well culture plates. One hundred microliters of synchronous ring stage cultures with 0.3% parasitemia and 2% hematocrit were added. Test plates were incubated for 72 h at 37°C under mixed gas (90% N_2, 5% CO_2, and 5% O_2). After 72 h of incubation, the plates were harvested and stored at −30°C for 24 h. Subsequently, the inhibition of the parasite was determined through a parasite lactate dehydrogenase (pLDH) assay performed by reading the absorbance at 650 nm using Multiskan Skyhigh microplate spectrophotometer (Thermo Fisher Scientific, USA) (Wang et al., 2019). The experiment was conducted in triplicates.

Culture cell lines

The Huh7 was obtained from the Department of Biomedical Chemistry, Graduate School of Medicine, The University of Tokyo, Japan, while the HepG2, BHK-21, and Vero cell lines were obtained from the National Research and Innovation Agency (BRIN). The cell line was maintained in DMEM supplemented with 3.7 g of $NaHCO_3$ in Milli-Q, 10% heat-activated fetal bovine serum, and 1% penicillium streptomycin. The cell line was incubated at 37°C under a humidified 5% CO_2 atmosphere. About 80% of confluence can be used for cytotoxicity assay (Nurkanto et al., 2022).

Cytotoxicity assay

The compound was dissolved in DMSO and diluted in culture media. Serial dilutions of the compounds were tested to determine the 50% cytotoxicity concentration (CC_{50}) using the AlamarBlue test method (Räz et al., 1997). The Huh7, HepG2, BHK-21, and Vero cell lines were seeded at 3×10^4, 1×10^4, 5×10^3, and 1×10^4 in 96 well-plate clear bottoms with serial concentrations of compounds and incubated at 37°C under a humidified 5% CO_2 atmosphere for 44 h. After incubation, 10 µL of 0.5 mM resazurin was added to each well, and incubation was continued for 4 h. The results were read at an excitation wavelength of 530 nm and an emission wavelength of 595 nm on a Nivo microplate reader (PerkinElmer). CC_{50} counted duplicates using GraphPad Prism version 6.0.

In silico docking

Hardware and software

Computational study in this research was performed under Maestro Schrödinger 2021-2 (Schrödinger, New York, NY, USA) software on Personal Computer Dell WorkStation, Linux Ubuntu 20.04.3 LTS OS, Intel® Xeon(R) W-2223 CPU @ 3.60GHz octa-core; RAM 16 GB and GPU NVIDIA Quadro P2200.

Preparation of ligands and receptor

The 2D structure of the ligands was constructed with Chemdraw, then converted into 3D and optimized using OPLS4 forcefield by LigPrep integrated into Schrodinger 2021-2 as well as protonated with Epik at pH 7.4 to restore improper or missing bonds, assign protonation, possible ionization, and tautomer-

ic states (Choudhary et al., 2020; Ikram et al., 2015; Schrödinger Release, 2022a; Zubair et al., 2021). Meanwhile, the falcipain-2 protein (PDB ID: 3BPF) was prepared under the Protein Preparation Wizard module in Maestro Schrodinger 2021-2 by removing the residual solvent, optimizing hydrogen bond, and protonated using ProtAssign and PROPKA also adding partial charge with OPLS4 forcefield (Madhavi Sastry et al., 2013; Olsson et al., 2011; Schrodinger Release, 2022b).

Molecular docking

The docking protocol was validated by redocking the native ligand on the binding site position, then the docked pose of the native ligand was used to calculate the Root Mean Square Deviation (RMSD) value. The docking protocol was valid if the RMSD value <2 Å (Henever et al., 2009). The molecular docking process was carried out in rigid receptors and flexible ligand conditions on extra precision (XP) mode by Glide. Subsequently, molecular mechanics-generalized Born surface area (MM-GBSA) was calculated for scoring the docked pose of the ligand to identify which ligands have the best binding affinity (Friesner et al., 2006; Genheden and Ryde, 2015; Schrodinger Release, 2022c; 2022d).

Statistical analysis

The data were presented as mean ± SD. The probit analysis was used to calculate the IC_{50} and CC_{50} values using GraphPad Prism Version 6.0 (GraphPad Software, San Diego, CA, USA).

RESULTS AND DISCUSSION

Isolation of antimalarial compound

A bioassay-guided fractionation led to the isolation and structure elucidation of the antimalarial compound from *A. sericicarpus* leaves. Compound 1 was isolated as a pale-yellow powder. The molecular formula was determined to be $C_{20}H_{22}O_4$ from its HRESIMS (m/z 327.1573 $[M+H]^+$). The IR spectrum showed absorption bands for a hydroxy group at 3327 cm^{-1}, a conjugated carbonyl at 1632 cm^{-1}, and an aromatic ring at 1448 cm^{-1}. The UV spectrum showed maximum absorptions at 314.5 nm and 277.5 nm in MeOH, indicating a chalconoid structure.

Table 1. ^1H (400 MHz) and ^{13}C (100 MHz) NMR data for compound **1** in acetone-d6.

Position	δ_H (mult, J in Hz)	δ_C	HMBC
1	-	132.81	-
2	7.00 (d, J=2.2 Hz, 1H)	130.47	C6, C4
3	-	128.56	-
4	-	154.03	-
5	6.73 (d, J=8.0 Hz, 1H)	115.65	C3, C1
6	6.92 (dd, J=8.4, 2.6 Hz, 1H)	127.39	C2, C4
α	3.22 (t, J= 6.0 Hz, 2H)	40.44	Cβ, C1, C=O
β	2.89 (t, J= 6.0 Hz, 2H)	29.12	Cα, C2, C6, C1, C=O
C=O	-	205.22	-
1’	-	113.90	-
2’	12.83 (s, OH)	166.28	C3’, C1’, C4’
3’	6.32 (d, J=2.4 Hz, 1H)	103.56	C5’, C1’
4’	-	165.50	-
5’	6.41 (dd, J=8.6, 2.4 Hz, 1H)	108.73	C3’, C1’
6’	7.81 (d, J=8.0 Hz, 1H)	132.13	C4’, C2’, C=O
1”	3.29 (m, J=8.0 Hz, 2H)	31.98	C2”, C3, C2, C4
2”	5.31 (td, J=7.5, 2.1 Hz, 1H)	123.91	-
3”	-	133.67	-
4”	1.69 (s, 3H)	25.88	C5”, C2”
5”	1.69 (s, 3H)	17.81	C4”, C2”

The ^{13}C NMR spectrum (see Table 1 and supplementary data) revealed the presence of 20 signals, including those for one carbonyl group (δ 205.22), three methylene carbons (δ 29.12, 31.98, and 40.44), and two methyl groups (δ 17.81 and 25.88), corresponding to a prenylated chalcone.

The ^{1}H NMR spectrum (Table 1) revealed the presence of a sharp singlet at δ 12.83, attributed to a chelated phenolic proton, and a set of signals at δ 5.31 (1H, *J*=7.5 Hz), δ3.29 (2H, *J*=8 Hz), and δ 1.69 (*s*, 6H) suggesting the presence of 3,3-dimethylallyl moiety. The presence of two sets of doublet of doublets, and two sets of doublets with ABX spin system suggests the presence of two sets of protons with *ortho* and *meta* arrangement on rings A and B. The presence of two triplets at δ 2.89 and δ 3.22 with the integration of 2H and coupling constant of 6 Hz each, and the absence of typical trans-olefinic protons of a chalcone in the down-field region suggesting compound 1 is a dihydrochalcone. The assignment of the substituents was supported by correlations observed in HMQC and HMBC. Compound 1 was identified as 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-en-1-yl) phenyl] propane-1-one (Fig. 1). The spectral data were comparable with licoagrochalcone A, or n 3-(3,3-dimethylally)-4,2′,4′-trihydroxylchalcone, which was previously reported in *Glycyrrhiza glabra*, *Dorstenia elliptica*, *Erythrina abyssinica*, *Lespedeza crytobotrya*, and *Glycyrrhiza inflata* (Asada et al., 1998).

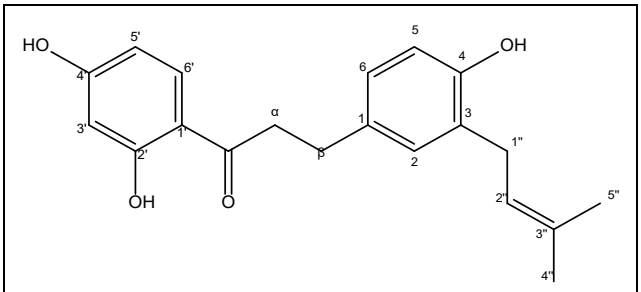


Figure 1. Structure of compound **1**.

LDH antimalarial activity and cytotoxicity

Genus *Artocarpus* showed potency as an antimalarial source, whereas numerous studies demonstrated antimalarial compounds isolated from this genus (Hafid et al., 2012; Hidayati et al., 2020; Widyawaruyanti et al., 2007). The preliminary results showed that dichloromethane extract of *A. sericicarpus* leaves exhibited antimalarial activity. Therefore, this study was conducted to isolate and identify antimalarial compounds from the dichloromethane extract of *A. sericicarpus* leaves.

The fractionation of the dichloromethane extract of *A. sericicarpus* leaves resulted in two fractions (A and B). The result of antimalarial activity showed that Fraction B was more active than Fraction A. The fractionation was continued for Fraction B to give five fractions (B1-B5). Those fractions were screened at a concentration of 10 µg/mL, and Fraction B5 highly inhibited *Plasmodium falciparum* growth for 83% inhibition compared to other fractions, which performed less than 50% of inhibition. The IC_{50} value of B5 was then determined. Purification of fraction B5 yielded two fractions (B5.1 and B5.2). Further purification was carried out for B5.2 considering the fraction amount besides the IC_{50} value. Three more fractions (B5.2.1, B5.2.2, and B5.2.3) were obtained from the separation of B5.2, and finally, compound **1** was obtained from the purification of B5.2.3. The results of LDH antimalarial activity are shown in Table 2. Based on the IC_{50} value of fractions and compound **1**, it was found that fraction B was the most active among others. Previous studies reported antimalarial criteria of antimalarial substances, which included active substances if the IC_{50} value is less than 10 µg/mL, moderate if the IC_{50} value is in a range of 10-50 µg/mL, low activity if the IC_{50} value in a range of 50-100 µg/mL, and inactive if IC_{50} value more than 100 µg/mL (Basco et al., 1994). Based on the criteria, Fraction B, Fraction B5, and Fraction B5.2.3 were indicated as active antimalarial substances. Meanwhile, Fraction B5.2 and compound **1** were included in moderate antimalarial substances. Compound **1** had an IC_{50} value of 11.35 ± 0.02 µg/mL (34.80 µM). The data showed that most fractions performed higher activity than compound **1**. This phenomenon usually occurs in natural product medicine, possibly due to the synergistic action of substances contained in fractions (Caesar and Cech, 2019). This fact suggests that compound **1** is not the most active substance that took a role in the antimalarial activity, meaning that a high opportunity exists to isolate more active compounds from these fractions.

Compound **1** belongs to the dihydrochalcone compound group. The dihydrochalcone compounds were reported to have anticancer, antidiabetic, antimicrobial, antioxidant, anti-inflammatory, hepatoprotective, antileishmanial activity, anti-HIV, anti-herpes simplex type-2, cardioprotective, anticholinesterase and anti-butyrylcholinesterase activity (Stompor et al., 2019). Several dihydrochalcone compounds isolated from *A. altilis* were reported to have antimalarial activity, namely 1-(2,4-dihydroxyfenyl)-3-(8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-yl-5-benzopyran)-1-propanone, 1-(2,4-dihydroxyfenyl)-3-{4-hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-dibenzo (b, d) pyran-5-il}-1-propanone, 2-geranyl-2',3,4,4'-tetrahydroxydihydrochalcone (Wang et al., 2007), 1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone (Hidayati et al., 2020). Nevertheless, the antimalarial activity of dihydrochalcone was given

less attention. This study is believed to be the first reported antimalarial dihydrochalcone compound isolated from *A. sericicarpus* leaf extract.

Numerous studies have reported that mostly prenylated flavonoids exhibited antimalarial activity (Boonphong et al., 2007; Bourjot et al., 2010; Hidayati et al., 2020; Widyawaruyanti et al., 2007). Prenyl-moiety supported the backbone compound more lipophilic, which led to its high affinity to the cell membrane. Thus, the prenylation enhances the compounds' bioactivity (Chen et al., 2014). Based on this data, the prenylation of compound **1** possibly takes a role in its antimalarial activity. Furthermore, the attachment of hydroxy groups enhanced the activity as well. Even though the antimalarial activity of compound **1** was moderate, the dihydrochalcone compound showed high potency. Dihydrochalcones are naturally occurring α, β-unsaturated carbonyl compounds, which are key precursors for the biosynthesis of other flavonoids for the synthesis of many biologically valuable heterocyclic compounds (Ardiansah, 2019; Stompor et al., 2019). Dihydrochalcone can be used as a scaffold for drug development. Modification of compound **1** will possibly be done synthetically to improve the compound affinity and increase the bioactivity.

The cytotoxicity of compound **1** was conducted against several cell lines as well. The result showed that compound **1** exhibited a CC_{50} value in a range of 34.35-75.86 µg/mL (Table 3). The criteria of cytotoxicity activity for botanical or crude extracts, according to The American National Cancer Institute USA (NCI), is IC_{50} <20 µg/mL or 10 µM upon 48 h or 72 h incubation (Boik, 2001). Based on the criteria, compound **1** could be considered a non-cytotoxic substance. The Selectivity Index (SI) values of compound **1** were evaluated to determine whether further work could be continued. SI was defined as the ratio of the toxic concentration of a sample against its effective bioactive concentration. Several publications reported many variations of the acceptance criteria of SI. A SI value ≥10 was recommended for the bioactive sample. If the SI value is between 1 and 10, re-evaluating using other biosystems is advocated for confirmation (Indrayanto et al., 2021). Compound **1** showed SI values in the range of 3.02-6.68 (Table 3); further study needs to be conducted to re-evaluate the toxicity or modification of structure, possibly synthetically done to decrease the toxicity.

In silico molecular docking

The genome of *P. falciparum* contains 33 open reading frames that encode four cathepsin L-like papain-family proteases known as falcipain that belong to the cysteine proteases related to the erythrocyte invasion. One of the falcipain receptors that can be used as an antimalarial target is falcipain-2. The disruption of falcipain-2 leads to decreased hemoglobin hydrolysis in trophozoites, as reported by the accumulation of undigested hemoglobin in the food vacuole (Rosenthal et al., 1988; Sijwali and Rosenthal, 2004). Molecular docking techniques could be used to identify potential compounds that actively inhibit falcipain-2 receptors.

Table 2. Antimalarial activity of fractions and compound 1.

Sample	IC_{50} (µg/mL)	Classification
Fraction A	2.35 ± 0.07	Active
Fraction B	2.11 ± 0.05	Active
Fraction B1	NA	NA
Fraction B2	NA	NA
Fraction B3	NA	NA
Fraction B4	NA	NA
Fraction B5	7.02 ± 0.03	Active
Fraction B5.1	11.71 ± 0.17	Moderate
Fraction B5.2	13.04 ± 0.02	Moderate
Fraction B5.2.1	NA	NA
Fraction B5.2.2	9.19 ± 0.01	Active
Fraction B5.2.3	5.32 ± 0.02	Active
Compound 1	11.35 ± 0.02	Moderate
Chloroquine diphosphate	0.008 ± 0.05	Highly active

Data represented as mean ± SD of triplicates.

Table 3. Cytotoxicity of compound **1** against several cell lines.

Cell line	Compound 1			Chloroquine diphosphate		
	CC_{50} (µg/mL)	CC_{50} (µM)	SI (CC_{50}/IC_{50})	CC_{50} (µg/mL)*	CC_{50} (µM)	SI (CC_{50}/IC_{50})
Huh7	51.06 ± 0.030	156.44	4.50	93.73 ± 0.15	181.70	>100
HepG2	62.64 ± 0.020	191.92	5.52	>100	>193.85	>100
BHK-21	34.35 ± 0.020	105.24	3.02	90.52 ± 0.24	175.47	>100
Vero	75.86 ± 0.002	232.42	6.68	>100	>193.85	>100

Data represented as mean ± SD of duplicates.

Table 4. Docking results of selected compounds on falcipain-2 receptor.

	E64 (Native ligand)	Chloroquine	Compound 1
MMGBSA (kcal/mol)	-55.59	-39.62	-40.2

Molecular docking is a drug design tool widely used to predict binding interaction between ligands and receptors. Even though docking analysis has been used in extensive drug development, the procedure must be validated. The docking protocol of falcipain-2 in this research was validated by internal validation through RMSD calculations. According to Fig. 2, the superimpose of the docked result and experimental pose of E64 as a native ligand showed a similar binding mode with an RMSD value of 1.3266 Å, which indicates the docking protocol was valid (Castro-Alvarez et al., 2017; Ramírez and Caballero, 2018).

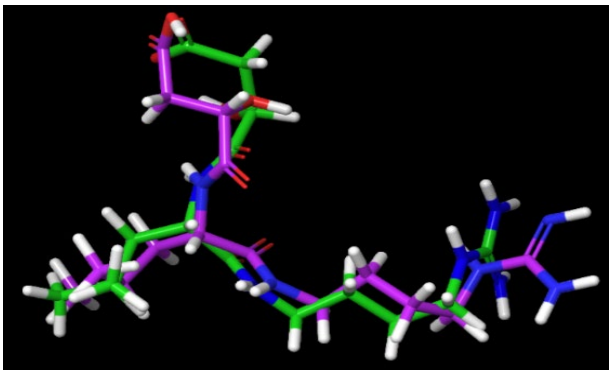


Figure 2. Superimpose of docked native ligand (E64) depicted in green; experimental poses in purple (RMSD = 1.3266 Å).

Docking's study determined the binding affinity based on molecular mechanics-generalized born surface area (MMGBSA) value, with a more negative value indicating a stronger binding (Choudhary et al., 2020). MMGBSA values exhibit E64 has the strongest binding affinity, then compound **1**, and chloroquine (Table 4). These results were also supported by molecular interactions of the ligands against falcipain-2 receptor (Table 5 and Fig. 3). The E64 ligand could bind to the falcipain-2 active sites (Gln36, Ser41, Cys42, Asn81, His174). Meanwhile, chloroquine possesses similar results. On the other hand, compound **1** showed the capability to bind to the falcipain-2 active sites except for Gln36. Even though the binding affinity of compound **1** was not better than E64, the capability to bind to the falcipain-2 active sites predicted compound **1** might inhibit falcipain-2 activity, which has an impact on the disruption of the life cycle of the *P. falciparum* parasite.

CONCLUSION

The study suggested that *A. sericicarpus* is a potential source of novel antimalarial substances. Dihydrochalcone compound identified as 1-(2,4-dihydroxyphenyl)-3-[4-hydroxy-3-(3-methylbut-2-en-1-yl) phenyl] propan-1-one has been isolated from *A. sericicarpus* leaves extract reported for the first time from this plant and as well as its antimalarial activity. The compound showed the ability to bind the falcipain-2 active sites on *in silico* investigation.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Table 5. Interactions of selected compounds against amino acid residues of falcipain-2 binding site.

	E64 (Native ligand)	**Chloroquine**	**Compound 1**
Amino acid residues	**36-Gln**	**36-Gln**	
	40-Gly	40-Gly	40-Gly
	41-Ser	**41-Ser**	**41-Ser**
	42-Cys	**42-Cys**	**42-Cys**
	43-Trp	43-Trp	43-Trp
	78-Tyr		
		80-Cys	80-Cys
	81-Asn	**81-Asn**	**81-Asn**
	82-Gly	82-Gly	82-Gly
	83-Gly	83-Gly	83-Gly
	84-Leu	84-Leu	84-Leu
	85-Ile	85-Ile	85-Ile
			86-Asn
		236-Phe	236-Phe
	234-Asp	234-Asp	234-Asp
	149-Ser	149-Ser	149-Ser
	150-Val		
		171-Gln	
	172-Leu	172-Leu	172-Leu
	173-Asn	173-Asn	173-Asn
	174-His	**174-His**	**174-His**
	175-Ala	175-Ala	175-Ala

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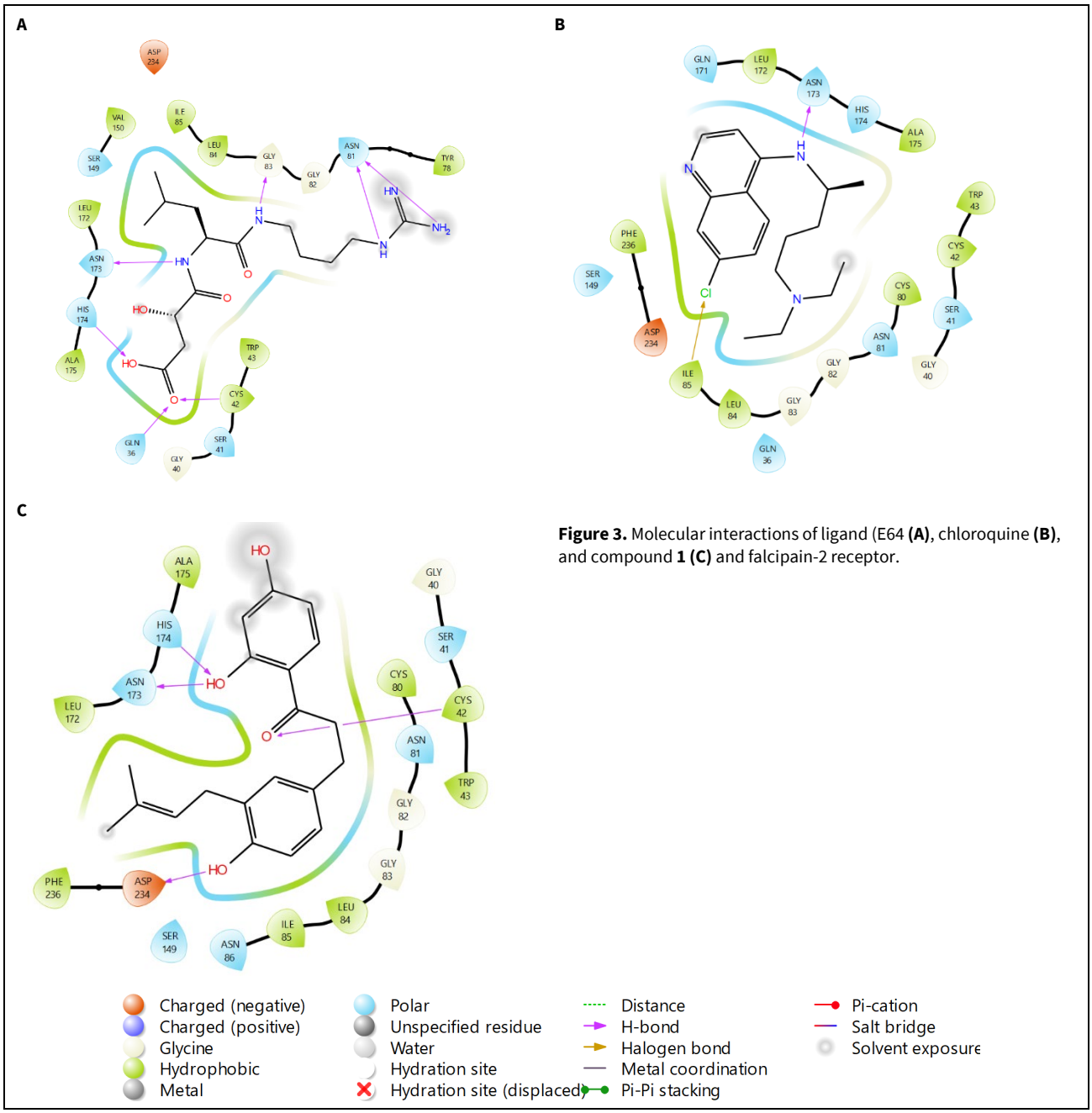


Figure 3. Molecular interactions of ligand (E64 **(A)**, chloroquine **(B)**, and compound **1 (C)** and falcipain-2 receptor.

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AUTHOR CONTRIBUTION:

Contribution	Tumewu L	Ilmi H	Saputri RD	Sari DK	Permanasari AA	Nisa HK	Maulana S	Tjahjandarie TS	Tanjung M	Osman CP	Ismail NH	Widyawaruyanti A	Hafid AF
Concepts or ideas	x							x	x		x	x	x
Design	x							x	x		x	x	x
Definition of intellectual content	x							x	x		x	x	x
Literature search	x	x		x			x			x			x
Experimental studies	x	x	x	x	x	x	x		x				x
Data acquisition	x	x	x	x	x	x							x
Data analysis	x	x		x			x		x	x			x
Statistical analysis	x	x		x									x
Manuscript preparation	x	x		x			x			x			x
Manuscript editing	x	x	x	x	x	x	x	x	x	x	x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	x	x	x	x

Citation Format: Tumewu L, Ilmi H, Saputri RD, Sari DK, Permanasari AA, Nisa HK, Maulana S, Tjahjandarie TS, Tanjung M, Osman CP, Ismail NH, Widyawaruyanti A, Hafid AF (2023) Antimalarial dihydrochalcone isolated from *Artocarpus sericicarpus* Jarret leaves and *in silico* investigation against falcipain-2 protein. J Pharm Pharmacogn Res 11(5): 797–809. https://doi.org/10.56499/jppres23.1661_11.5.797

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Supplementary data

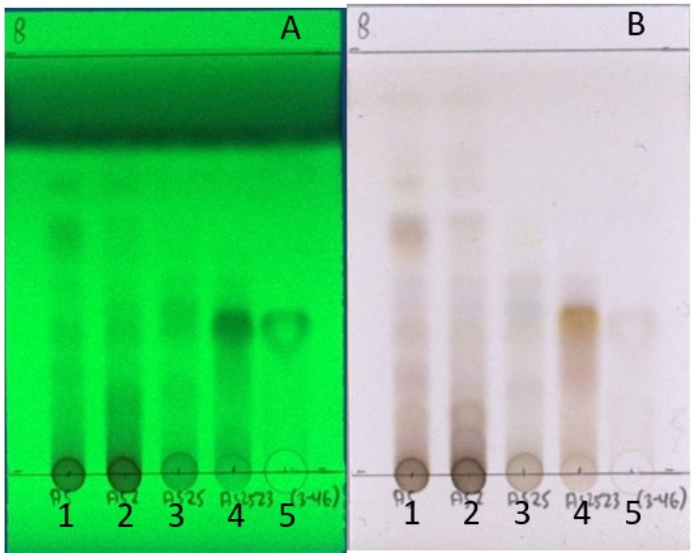


Figure S1. Thin Layer Chromatogram profile of dichloromethane extract **(1)**, fraction B **(2)**, fraction B5 **(3)**, fraction B5.2.3 **(4)**, compound 1 **(5)**, using silica gel GF_{254} as stationary phase and hexane-ethyl acetate 4:1 v/v as mobile phase. The plate was observed under UV 254 nm **(A)**, sprayed using H_2SO_4 10%, and heated at 105°C for 5 min **(B)**.

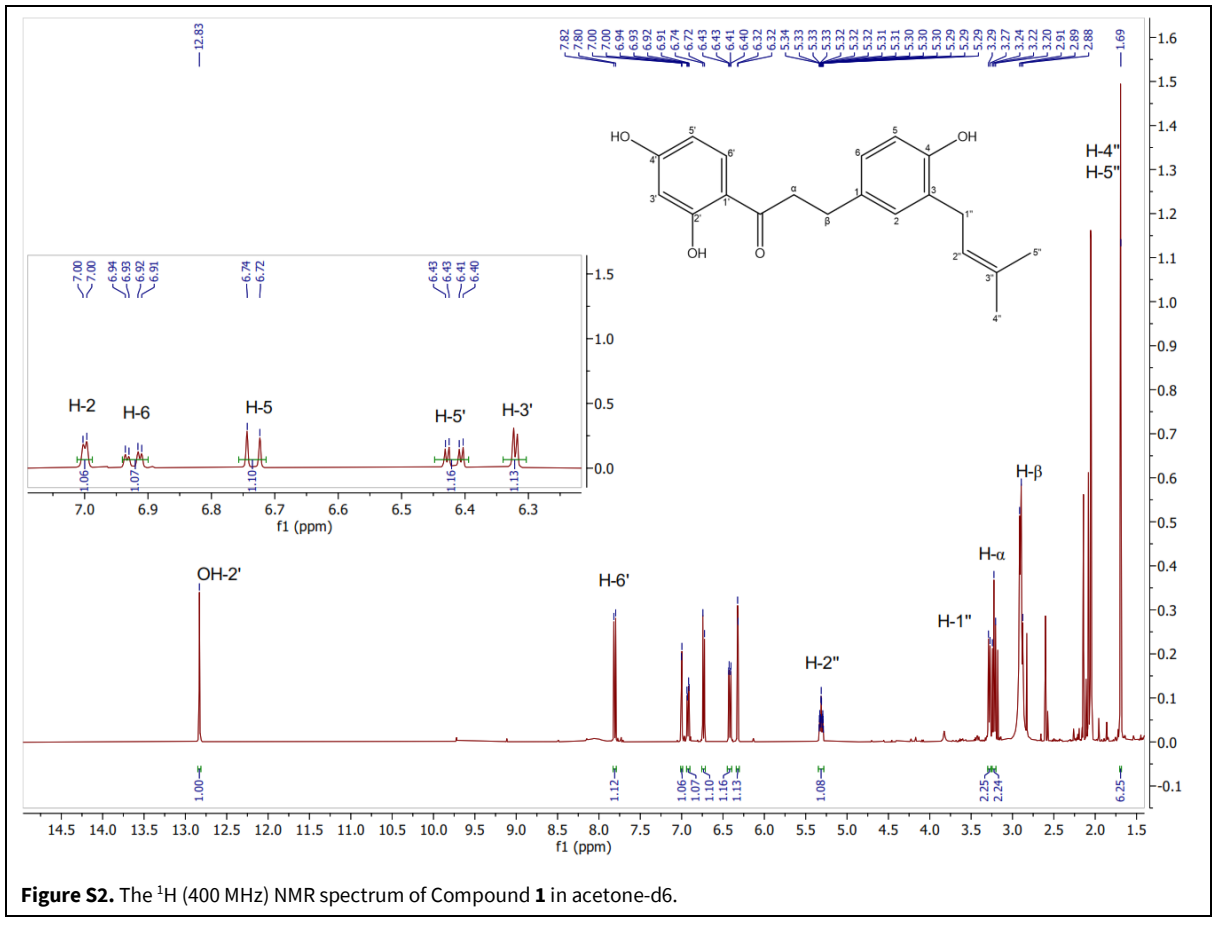


Figure S2. The ^1H (400 MHz) NMR spectrum of Compound **1** in acetone-d6.

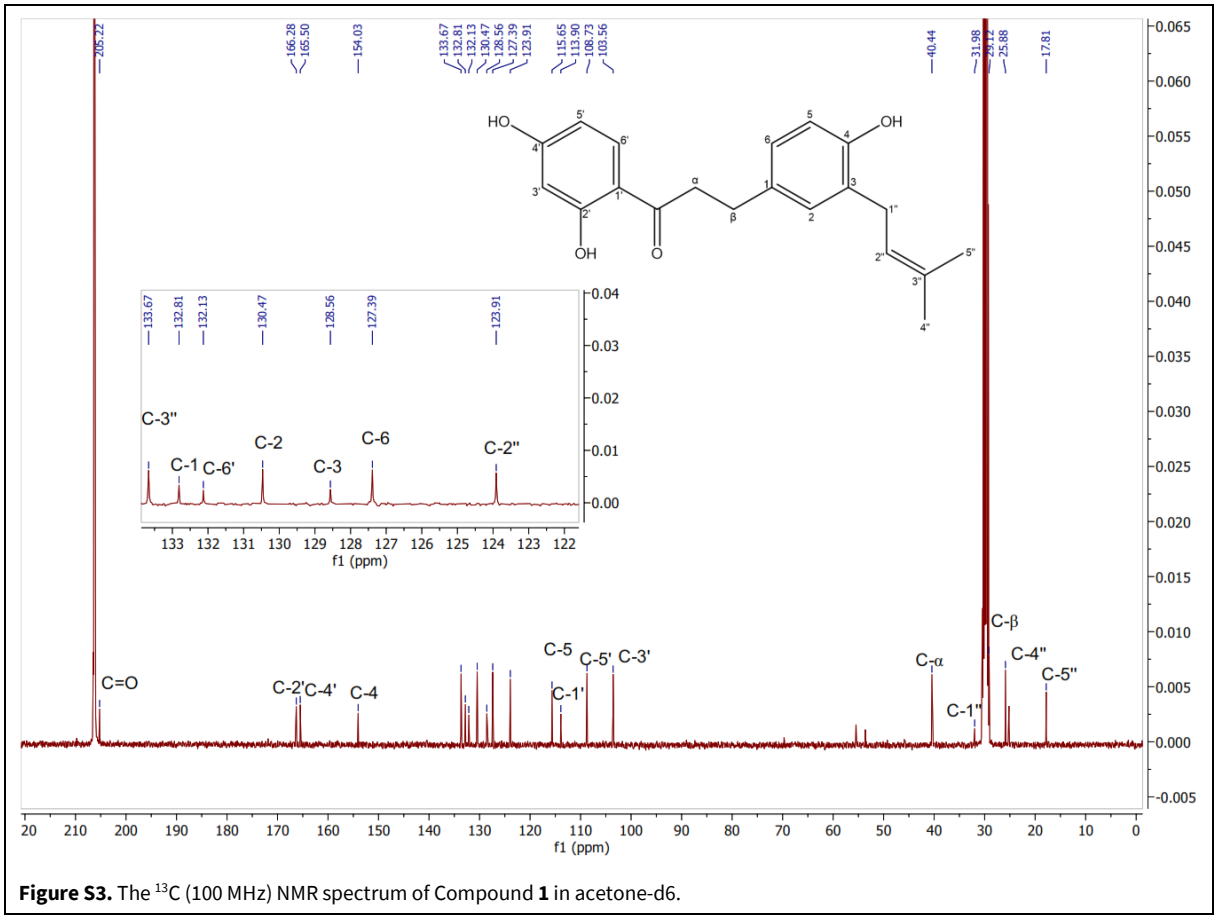


Figure S3. The ^{13}C (100 MHz) NMR spectrum of Compound **1** in acetone-d6.