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Research Article

Antimalarial Activity of Flavonoid Compound Isolated from Leaves of *Artocarpus altilis*

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ABSTRACT

Introduction: *Artocarpus altilis* leaves extract has previously been reported as a potential antimalarial drug. Inhibition concentration (IC_{50}) against *P. falciparum* and effective dose values (ED_{50}) against *P. berghei* have been reported at 1.32 µg/ml and 0.82 mg/kg, respectively. The aim of this study is to identify the active compound from the ethanol extract of *A. Altilis* leaves against *P. falciparum*. **Materials and Methods:** The isolation of the active compound from the ethanol extract of *A. altilis* were conducted using chromatography methods, and the chemical structure of the isolated compounds was determined based on NMR and MS spectra data. Antimalarial assay was determined using microscopic method against *P. falciparum* 3D7 and molecular docking studies was performed using Molegro Virtual Docker version 5.5 program. **Results:** A flavonoid compound, class of dihydrochalcone was finally isolated from *A. altilis* and identified as 1-(2,4-*dihydroxy phenyl*)-3-[8-*hydroxy*-2-*methyl*-2-(4-*methyl*-3-*pentenyl*)-2H-1-*benzopyran*-5-yl]-1-*propanone* (Compound-1). Antimalarial activity test revealed that the compound strongly inhibited *P. falciparum* growth, with IC_{50} value of 1.05 µM. An *in silico* study to determine the mechanism of action of the compound revealed the existence a 3.BPF receptor that possesses a cysteine protease inhibitor of falcipain-2. **Conclusion:** Compound-1 were isolated from the leaves of *A. Altilis* is a good candidate of new source in the development of antimalarial drugs. An animal study using this compound is recommended before a clinical trial.

Key words: *Artocarpus altilis,* Cysteine protease inhibitor, Dihydrochalcones, *P. falciparum* 3D7.

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INTRODUCTION

Malaria is a potentially fatal disease transmitted through the bite of female *Anopheles* mosquito and affects more than 106 countries worldwide. Mortality rates of malaria have increased over the past 20 years, primarily due to resistance of *P. falciparum* to antimalarial drugs, especially chloroquine, artemisinin and its derivatives.[1,2] Efforts are therefore needed to develop new antimalarial as that are effective, safe and have fewer side effects while also being cheap and easy to obtain, one potential source are compounds derived from plants.[3] Flavonoids are secondary metabolites that are widely found in plants, and the antimalarial activity of this group has been widely reported.[4]

Moraceae, a family of flowering plants, consists of 60 genera that include 1,400 species. The largest genus of the Moraceae family is *Artocarpus* which contains 50 species, 40 of which are in Indonesia. The genus *Artocarpus* is widely used in traditional medicines. Generally, *Artocarpus* species are rich in phenolic compounds. The extracts and metabolites of *Artocarpus*, especially leaves, barks, stems, and fruits, have been shown to be useful bioactive compounds. Several pharmacological studies on *Artocarpus* have proven its utilization for various diseases such as inflammation, malarial fever, diarrhea, diabetes, and tapeworm infections. *Artocarpus altilis*, which in Indonesia has a local name "sukun" referred to as breadfruit, is a tropical plant. The breadfruit tree produces fruit from March to September. The synonyms of *A. altilis* are *A. communis*, and *A. incises*.[5,6]

An ethanol extract of *A.altilis* leaves actively inhibited the growth of *P. falciparum in vitro* with IC_{50} values 1.32 µg/ml, and was highly active against *P. berghei in vivo* with ED_{50} values 0.82 mg/kg body weight. While stem bark extract from *A. altilis* showed a very good *in vivo* activity against *P. berghei*, and weak *in vitro* activity against *P. falciparum*.[7] The previous study showed that another species of genus *Artocarpus* reported that prenylflavonoid compounds isolated from *A. champeden* stem bark extract. *A. champeden* stem bark extract contains artocarpones A, artocarpone B, artoindonesianin E, heteroflavanone C, artoindonesianin R, heterophyllin, artoindonesianin A-2, cycloheterophyllin, and artonin. Heteroflavanone C had the most active inhibition against *P. falciparum* with IC_{50} values 1 nmol/L.[8] A prenylated chalcone, isolated from *A. champeden* stem bark extract, namely morachalcone A, was identified as a antimalarial active marker compound.[9] Leaves and stembark extract from another species, *Artocarpus heterophyllus* and *Artocarpus camansi,* also has been reported to have good antimalarial activity against *P. falciparum* and *P. berghei*.[7,8]

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While *A. altilis* leaves extract is efficacious against malarial parasite, their mechanism of action remains to be identified. This study was conducted to search for active antimalarial compounds contained in the leaves extract of *A. altilis*.

MATERIALS AND METHODS

General

NMR spectra were recorded on a JEOL ECS-400, using $CDCl_3$ as the solvent. The HPLC system also includes two LC-10AD pumps and a SCL-10A controller. An Agilent RP-18 XDB column 4.6 x 250 mm was eluted with CH_3CN-H_2O (7:3 v/v) at 2 mL/minute of flow rate. Vacuum Liquid Chromatography (VLC) on silica gel GF_{254} (Merck, Cat No. 1.07730.0500) and Thin Layer Chromatography (TLC) was carried out on silica gel 60 F_{254} (Merck, Cat No. 1.05715.0001) and RP-18 silica gel plate (Merck, Cat No. 1.15389.0001). The identification of TLC profile was performed using TLC Visualizer (Camag).

Plant material

The fresh leaves of *Artocarpus altilis* were collected from Purwodadi Botanical Garden, East Java, Indonesia in the rainy season, around September-December, and identification by a licensed botanist at Purwodadi Botanical Garden, East Java. A voucher specimen (No: B-107/IPH.06/AP.01/II/2020) of this raw material stored at the Herbarium of the Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

Isolation and identification of active compounds

The ethanol extract was fractionated using vacuum liquid chromatography (VLC) with a gradient of n-hexane and ethyl acetate (100%-0%). Based on thin layer chromatography (TLC) profiles, several fractions with the same profile were combined and yielded 6 fractions. Each fraction was identified with TLC methods and tested for its antimalarial activity. Fraction that contain yellow spot on its TLC profile and showed inhibition against P. falciparum, was then separated by semi-preparative High Liquid Pressure Chromatography (HPLC) methods. The semi-preparative HPLC system was run on a Shimadzu, LC-06, included two LC-10AD pumps and SCL-10A controller, with an semi preparative colomn; Agilent Eclipse XDB-C18, 5 µm (9.4 x 250 mm). Fraction were eluted in a methanol:water (8:2, v/v) mixture at flow rate of 1 mL/min, which obtained three isolates.

The active isolate profile was analyzed using TLC and HPLC. TLC was analyzed using a silica gel 60 F_{254} plates, scanned by TLC Visualizer, visualized on UV 254 nm and 366 nm, and sprayed with H_2SO_4:water (1:9, v/v) to highlight the flavonoid compounds. The HPLC analysis system included two LC-10AD pumps and SCL-10A controller, with an analytical colomn; LiChrospher ®100 RP-18 analytical column, 5 µm, 4.6 x 250 mm. Compounds were eluted in a methanol:water (8:2, v/v) mixture at flow rate of 0.7 mL/min. The chemical structure of each isolate was determined using a Nuclear Magnetic Resonances (JEOL ECS 400) with $CDCl_3$ as the solvent. The spectra were then analyzed using the MNova program. Mass spectra were identified by LC system coupled with Agilent Q-TOF/MS -MS-MS for HRESIMS analysis using methanol:water (8:2, v/v), with Agilent Zorbax Extend-C18 column, 1.8 µm, 2.1 x 50 mm

In vitro cultivation of Plasmodium falciparum

P. falciparum strain 3D7 was obtained from the Malaria Laboratory at The Centre of Natural Product Medicine Research and Development, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia. The culture was established using the method described by Trager and Jensen with some modifications.[10] Parasite were grown in fresh human erythrocytes (human type O red blood cell) at 5% haematocrit in complete RPMI-1640 medium supplemented with 10% (v/v) human type O-positive serum, 25 mM Hepes buffer, 50 µg/mL hypoxanthine, 2 mg/mL $NaHCO_3$, and 10 µg/mL gentamycin. The culture was incubated at 37°C in a modified candle jar. Parasitemia was determined by the examination of Giemsa's stained thin blood smears of infected erythrocytes. Human RBCs and serum were received from The Indonesian Red Cross, Surabaya, Indonesia.

In vitro antimalarial assay

Antimalarial assay was conducted using giemsa stained slide method.[11] Ten milligrams of tested compound and chloroquine was each diluted in 1 mL DMSO. The sample was then serially diluted in RPMI-1640 medium and prepared in dilution at a concentration of 0.01, 0.1, 1, 10, and 100 µg/mL in a 24 well plate. 500 µL parasite culture (±1% parasitemia, 5% hematocrit) was added to each well and incubated for 48 hours at 37°C. After incubation, thin blood smears were made and stained using 10% (v/v) Giemsa dye to showed infected red blood cell. The percentage of parasitemia was determined by counting infected erythrocytes per 1000 total erythrocytes under a light microscope. The percentage of growth inhibition was calculated using the following equation:

$$100\% - \left(\frac{Xe}{Xk} \times 100\%\right)$$

Where Xe is the average parasitemia of the experimental group, and Xk is the average parasitemia of negative control.

The IC_{50} values were calculated by applying four parameters logistic regression curve to the dose-response data using GraphPad Prism 7.0 software (GraphPad Co. Ltd., San Diego, CA, USA).

Criteria of antimalarial activity *in vitro*

In line with WHO guidelines and basic criteria for antiparasitic drug discovery activities of extracts were classified into four classes according to their IC_{50} values: high activity (IC_{50} ≤5 µg/ml); promising activity (5 µg/ml < IC_{50} ≤15 µg/ml); moderate activity (15 µg/ml < IC_{50} ≤50 µg/ml); weak activity (IC_{50} >50 µg/ml). A pure compound is defined as highly active when its IC_{50}≤1 µg/ml.[12]

Docking analysis

The molecular structure of the falcipain-2 enzyme was downloaded from the RCSB protein data bank website (http://www.rcsb.org/pdb/home/home.do). In this study, the falcipain-2 was chosen with the code PDB: 3BPF. This receptor was chosen because showed strongest interaction with compound-1. The ligand (was prepared by making 2D, and 3D structures of compound-1 using the ChemBioOffice program Ultra 11.0 and its energy was minimized using MMFF94. The ligand with the minimum energy that has been measured is then saved in the form of anmol2 {SYBYL2 (*.Mol2)}. The doctoring process of the enzyme falcipain-2 pdb.3BPF uses a Molegro Virtual Docker Version 5.5 program. The results obtained in the form of the Rerank Score (RS) value, which is the energy needed in the ligand-receptor interaction process, and from these values antimalarial activity can be predicted.[13]

RESULTS

Isolation and identification of active compound

An ethanol extract of *A. altilis* was fractionated by the VLC method using the gradient solvent of n-hexane and ethyl acetate (100%-0%) yielded six fractions (fraction 1-6). Identification with TLC method showed that fraction-3 gave the yellow spot that indicated a flavonoid compound. Fraction-3 was further separated by semi-preparative HPLC using acetonitrile:water (7:3 v/v) and yielded five isolates (isolate 1-5).

In vitro **antimalarial assay** showed that isolate-2 inhibited *P. falciparum* growth with IC_{50} of 0.48 µg/ml. Isolate-2 was analysed by TLC method using silica gel 60 RP-18 F254, mobile phase acetonitrile:water (7:3 v/v)= 7:3 v/v, scanned on UV 254 and 366 nm, and sprayed with a specific reagent for flavonoid compound. The results showed that isolate-2 had the yellow spot, typical of a flavonoid group, with Rf 0.4. Analysis of isolate-2 with HPLC method (Figure 1), at concentration 0.5 mg/ml, using isocratic solvent acetonitrile: water (7:3 v/v) showed that isolate-2 had a major peak, with retention time at 15.74 minutes. The UV spectrum of the peak showed maximum absorption with λ_{max} 248, 275, 300, and 315 nm (Figure 2). The UV spectra of most flavonoids consists of two major absorption, for band II in the range of 240-285 nm and band I in the range of 300-400 nm. In general terms the band II absorption may be considered as having originated from the A-ring benzoyl system and band I from the B-ring cinnamoyl system.[14] The UV spectrum of major peak showed a characteristic absorption peak of the flavonoid compound, class of dihydrochalcone.[15]

Isolate-2 was then identified based on proton and carbon signals in the ^{1}H and ^{13}C-NMR spectrum, HMBC, HMQC, and COSY. The ^{1}H NMR spectrum shows the presence of 2 aromatic rings, ring A and ring B. Ring A structure can be confirmed from the aromatic protons which showed ABX system at δ_H 6.32 (1 H, d, *J* = 2.4 Hz, H-3′), δ_H 6.35 (1 H, dd, 2.4, *J* = 8.4 Hz, H-5') and δ_H 7.58 (1H, d, *J* = 9.2 Hz, H-6'). In ring B the aromatic proton signal shows ortho-coupled at δ_H 6.62 (1 H, d, *J* = 8.0 Hz, H-6) and δ_H 6.72 (1 H, d, *J* = 8.0, Hz, H-5). Singlet methyl signals at δ_H 1.38 and 2 cis-olefenic proton doblets at δ_H 6.55 (1 H, d, *J* = 10.0 Hz, H-1") and δ_H 5.64 (1 H, d, *J* = 10.0 Hz, H-2"), and carbon quartener at δ_C 78.8 (C-3") indicates a 2.2-dimethylchromene ring which is substituted in ring B. A chelated hydroxyl group at at δH 12.74 (1H, s, 2'-OH), and two hydroxyl signal showed at aromatic ring at δH 7.25 (4'-OH) and δH 5.58 (4-OH). The methylene group signal at δ_H 3.10 (2H, q, H-α) and δ_H 2.97 (2H, m, H-β) substituted to C7 shows the correlation between ring A and ring B. The characteristics of chalcone pyrano-O-prenylated are shown by the methylene group signal at δH 3.1 (2H, q, H-alfa) and δH 2.97 (2H, m, H-beta) which have a correlation with carbonyl at δC 203.8. The prenyl group signal at δ_C 5.08 (1H, t, H-6"), δ_C 1.66 (3H, s, H-9") and δ_C 1.56 (3H, s, H-8") substituted with C6". The ^{13}C NMR spectrum of isolate-2 shows 25 well-separated signals for 25 carbon atoms, consisting of one conjugated carbonyl carbon at δ_C 203.8 (C=O), four oxyaryl carbon bond with two aromatic ring at δ_C 139.6 (C-3), δ_C 143.2 (C-4), δ_C 165.3 (C-2'), δ_C 162.3 (C-4'), one 2.2 dimethylcromen ring at δ_C 78.7 (C-3"), and one isoprenyl at δ_C 132.1 (C-7"). By conducting a references study on compounds that have been isolated from *A. altilis*, and comparing the NMR spectrum with the references, it is concluded that isolate-2 (Compound-1) is a flavonoid compound, class of dihydrochalcone, similar to [1-(2,4-*hydroxyphenyl*)-3-[8-*hydroxy*-2-*methyl*-2-(4-*metyl*-3-*penthenyl*)-2H-1-*benzopiran*-5-yl]-1-*propanone*].[16]

The molecular structur of Compound-1 has been confirmed it mass spectra which were identified by Agilent LC system coupled with Q-TOF/MS for HRESIMS analysis. HR-MS measurement revealed an ion peak $[M+H]^+$ at *m/z* 409.1998 (calculated 409.210), consistent with molecular formula of $C_{25}H_{30}O_5$

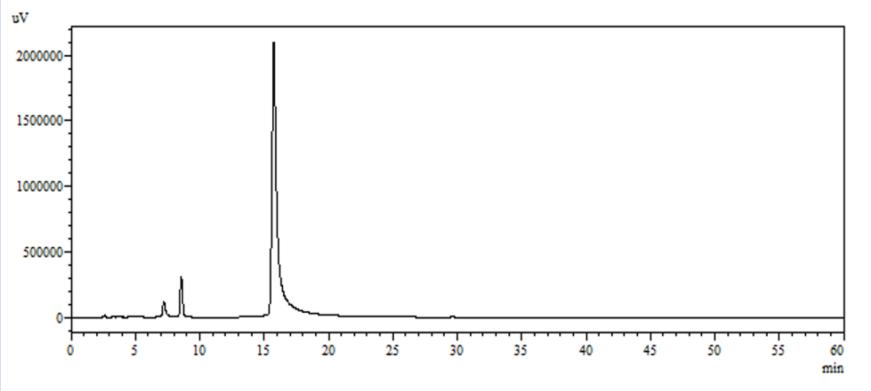

Figure 1: HPLC Chromatogram of compound 1.

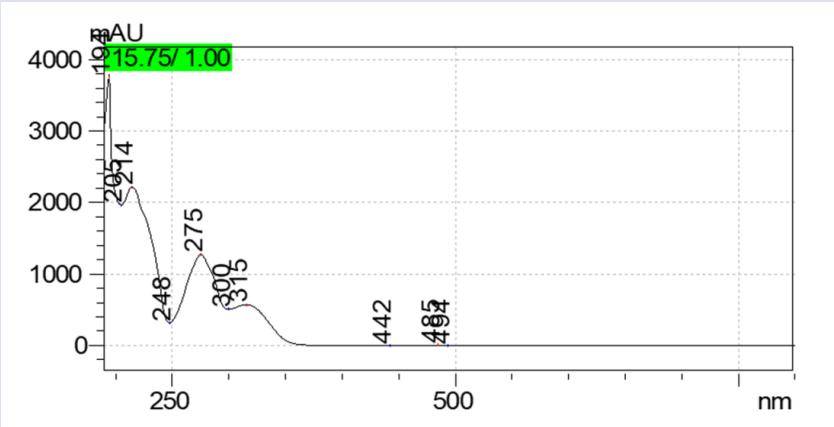

Figure 2: UV-VIS chromatogram of compound-1.

Compound-1

Compound-1 was identified as 1-(2,4-*dihydroxy phenyl*)-3-[8-*hydroxy*-2-*methyl*-2-(4-*methyl*-3-*pentenyl*)-2H-1-*benzopyran*-5-yl]-1-*propanone* (Figure 3).[16]

Yellow gum, *m/z* 409.2100, the 1H-NMR spectral data were identical to those in reference : ^{1}H-NMR ($CDCl_3$, 400 MHz) 1.66 (s), 1.56 (s), 1.38 (s), 1.79 (m), 2.09 (m), 2.97 (m), 3.10 (q), 5.08 (t), 5.58 (bs), 5.64 (d, *J* = 10 Hz), 6.32 (d, = 2.4 Hz), 6.35 (dd, *J* = 8.4, 2.4 Hz), 6.55 (d, *J* = 10 Hz), 6.62 (d, *J* = 8.0 Hz), 6.72 (d, *J* = 10.0 Hz), 7.25 (d, *J*= 9.2 Hz), 7.58 (d, *J*= 9.2 Hz) and 12.74 (s).

Antimalarial activity

In vitro antimalarial test was conducted using a synchronized *P. falciparum* strain 3D7, which is sensitive to chloroquine. This antimalarial activity test was conducted to determine the potential inhibition of *P. falciparum* growth in general, meaning that no inhibition was observed in certain phases. Chloroquine was use as a positive control.

Antimalarial assay of the Compound-1 (Table 1) *revealed that this compound inhibited the growth of P. falciparum with the* IC_{50} value of 0.48 µg/ml (calculated 1.05 µM). The IC_{50} value indicated that this compound possesses a potent antimalarial activity. This evidence suggests for further biochemical assay of the compound to determine the mechanism of action. The fact that the compound was originated from the leave extract also indicate its high availability and sustainability as we do not need to cut the tree source.

Docking analysis

To predict the mechanism of action, an *in silico study* was performed using the Molegro Virtual Docking (5.5) program to determine the possible interaction of compounds with the protein target. We evaluated several proteins from Protein Data Base, which have been reported to have an interaction with falcipain (www.rcsb.org). As a result, the isolated compound was predicted to have strong interaction with receptor 3BPF, falcipain-2, a protein that can be found in the food vacuole of *P. falciparum* involved in hemoglobin hydrolysis. The *in silico* study was performed by compari ng Compound-1 with drug references, chloroquine. The re-rank score of Compound-1 was -71.2844 kcal/mol, while the rerank score of choloroquine was -66.3610 kcal/mol. A lower value of the re-rank score indicates a stronger interaction to a receptor [13]. Hydrogen bond interaction was predicted between falcipain 2 and Compound-1 group with Gln 36* (2 bonds), Asn 173*, and Gly 83. In addition, van der Walls interactions were predicted between falcipain-2 and Compound-1 group with Gln 36, cys 39, Ser 41, Gly 40, Cys 42, Leu 172, Asn 173, Trp 206, His 174, and Val 152. It seems to contribute to the binding interaction of 3BPF and Compound-1 (Figure 4). While, chloroquine revealed hydrogen binding to Gly 83, Gln 36, Cys 42, and His 174, and steric van der Walls to Cys 42, Ser 41, and Gly 82 (Table 2). The interaction is described in the 3D profile (Figure 5).

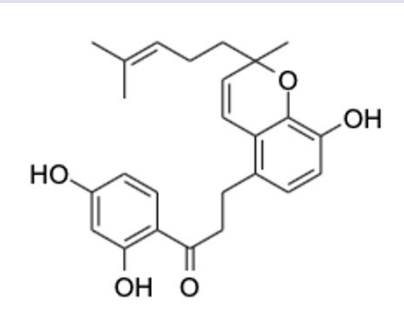

Figure 3: Molecular structure of compound-1: *1-(2,4-dihydroxyphenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone.*

Table 1: The IC_{50} value of all samples against *P. falciparum* 3D7.

Sample	IC_{50} (µg/mL)
Compound-1	0.43 ± 0.05
Chloroquine	0.004 ± 0.00

Data are reported as Mean ± SD.

Table 2: Hydrogen bond and van der Walls Interaction of Compound-1 and Chloroquine.

Hydrogen Bond		van der Walls	
Compound-1	Chloroquine	Compound-1	Chloroquine
Gln 36 (two bonds) Asn 173 Gly 83	Gln 36 Gly 83 Cys 42 His 174	Gln 36 Cys 39 Ser 41 Gly 40 Cys 42 Leu 172 Asn 173 Trp 206 His 174 Val 152	Cys 42 Ser 41 Gly 82

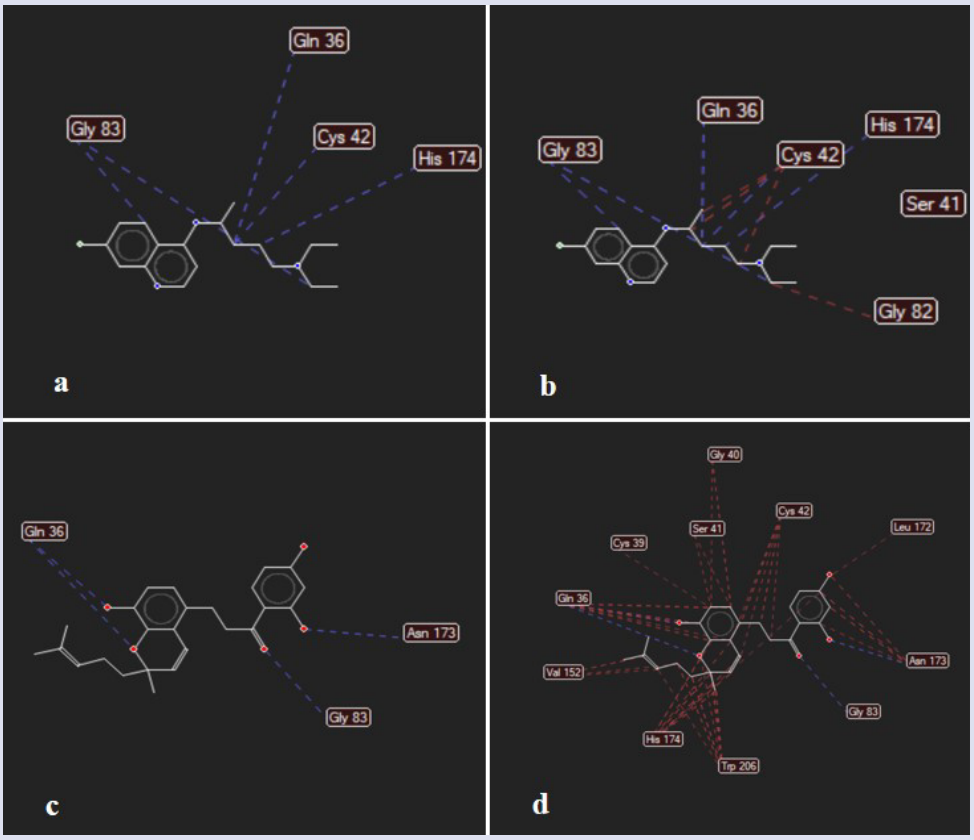

Figure 4: Hydrogen bond interaction (dashed blue-line) and Steric-Van der Walls bond interaction (dashed red-line) between Chloroquine (a and b) and Compound-1 on the active site of *P. falciparum* protein 3BPF (c and d).

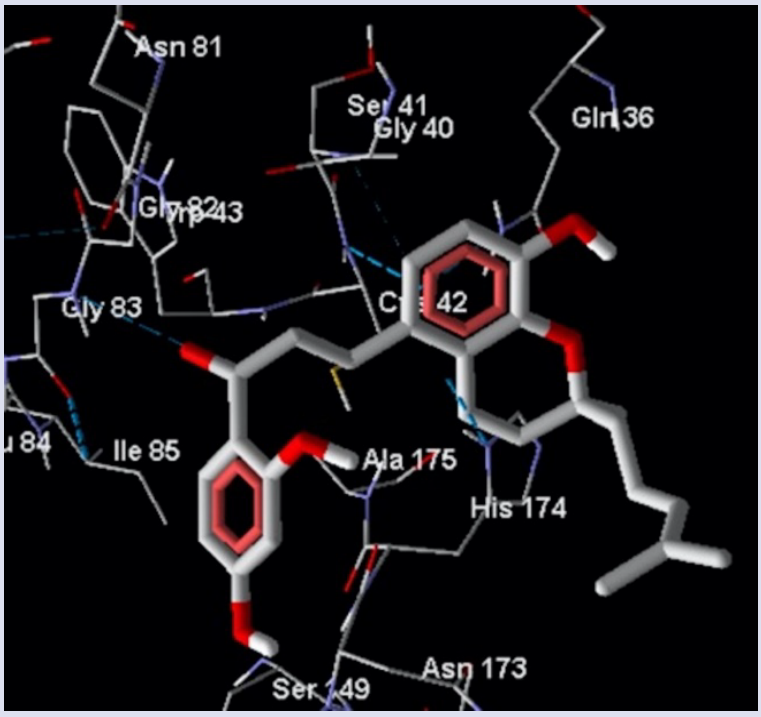

Figure 5: The 3D profile of Secondary Structure View of active compound-1 (green color) with 3BPF.PDB protein.

DISCUSSION

This research has identified the active antimalarial compound from ethanol extracts of *A. altilis* leaves. A flavonoid compound, class of dihydrochalcone known as 1-(2,4-*dihydroxy phenyl*)-3-[8-*hydroxy*-2-*methyl*-2-(4-*methyl*-3-*pentenyl*)-2H-1-*benzopyran*-5-yl]-1-*propanone* was identified. HR-MS measurement revealed an ion peak $[M + H]^+$ at *m/z* 409.1998 (calculated 409,210), consistent with the molecular formula of $C_{25}H_{30}O_5$. Lotulung et al. have also previously reported the isolation of compound-1 from the same plant.[16] Although this is a known compound, the antimalarial activity for this compound was firstly reported in this study. Compound-1 is classified as very active as an antimalarial with the IC_{50} value of 0.48 µg/ml (calculated at 1.05 µM). IC_{50} values indicate that this compound has potential antimalarial activity.[12]

Antimalarial activity of the dihydrochalcone have been widely reported. 2′,4′,6′-*trihydroxy*-4′-*methoxy dihydrochalcone* (asebogenin) from *Piper hispidum* and 2′,6′-*dihydroxy*-4′-*methoxydihydrochalcone* was reported to have potential antimalarial activity.[17,18] Two dihydrochalcone compounds were isolated from *Metrodorea stipularis* [1-(5,7-*dihydroxy*-2,2-*dimethylchroman*-6-yl)-3-(2,2-*dimethylchroman*-6-yl)*propan*-1 and 1-(5,7-*dihydroxy*-2,2-*dimethylchroman*-6-yl)-3-(1,1,4a-*trimethyl*-2,3,4,4,9-*hexahydro*-1*Hxanthen*-7-yl)*propan*-1] showed significant growth inhibition against parasite 3D7, Dd2, and W2 strains.[19] Carrol et al (2008) reported that prenylated dihydrochalcones from *Boronia bipinnata* inhibited the enzyme Hemoglobinase II in malaria parasites.[20] Hemoglobinase II, also known as plasmepsin II, is an aspartic protease that is found in the *P. falciparum*. The enzyme is involved in the digestion of hemoglobin from host cells within the acidic food vacuole of the parasite.[21] Based on this evidence, we then performed an *in silico* study to predict the mechanism of action of compound-1.

We evaluated several proteins from Protein Data Base which reported have an interaction with P. falciparum (www.rcsb.org) in food vacuole. The study showed that compound-1 have a strong interaction with 3.BPF protein, a facipain-2 receptor. It has been reported that Falcipain-2 is a cysteine protease that can be found in food vacuole of *plasmodium*.[22] This enzyme is a key member of the hemoglobin degradation pathway, a process that is required at erythrocytic stages of *P. falciparum* to obtain amino acids.[23,24] Falcipain-2 is an attractive target for drug discovery.[25,20] Nowadays, the search for potent falcipain-2 inhibitors is a hot topic in the search for potential malaria drugs.[26] Other studies have also shown that trophozoites falcipain-2 knock out cause a decrease in cysteine protease activity, accumulation of hemoglobin that cannot be degraded in food vacuoles, and increase the sensitivity of cysteine and aspartic protease inhibitors.[27] *In silico* study predicted that the target site of Compound-1 is in parasitic food vacuoles as a cysteine protease inhibitor. Based on our study, we concluded that prenylated dihydrochalcones acted by inhibiting the activity of Plasmepsin II enzyme, resulting in the inability of hemoglobins to be degraded into large fragment. Meanwhile, compound-1 acted by inhibiting the activity of falcipain-2 enzyme, hence disabling the degradation of large fragments into amino acids.

CONCLUSION

1-(2,4-dihydroxy phenyl)-3-[8-hydroxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-5-yl]-1-propanone were isolated from the leaves of Artocarpus altilis is a good candidate of new source in the development of antimalarial drugs. The in silico study predicted that the mechanism of action of this compound had a strong interaction with 3BPF, falcipain-2 receptor, as a cysteine protease inhibitor. Further study on animal model using this compound is also recommended before a clinical trial.

CONFLICTS OF INTEREST

The authors declare that they have no competing interest.

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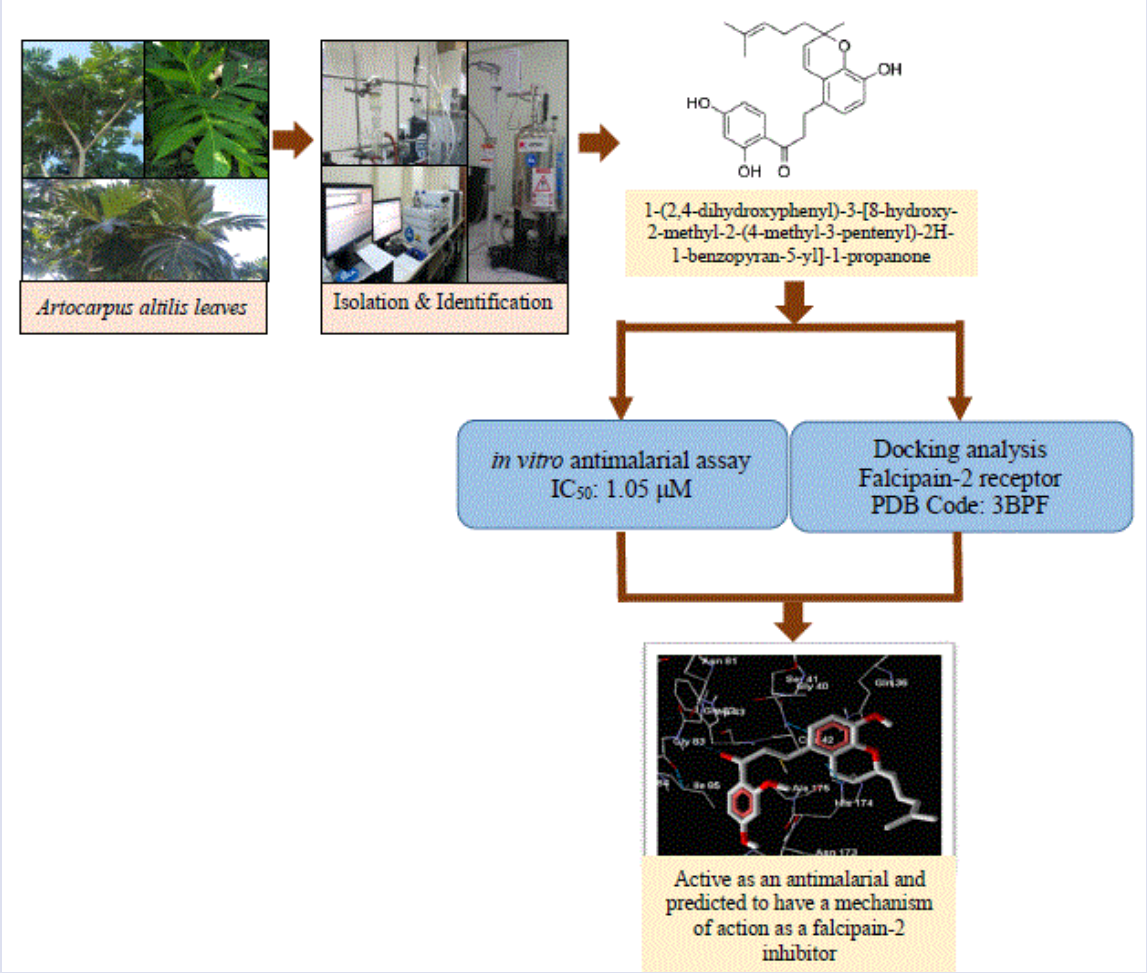
REFERENCES

1. WHO. World Malaria Report 2015. Geneva: Switzerland. World Health Organization. 2015.
2. Cowman AF, Healer J, Marapana D and Marsh K. Malaria: Biology and Disease. Cell. 2016;167(3):610-24.
3. Cui L, Mharakurwa S, Ndiaye D, Rathod PK, Rosenthal PJ. Antimalarial drug resistance: literature review and activities and findings of the ICEMR network. Am J Trop Med Hyg. 2015;93(3 Suppl):57-68.
4. Saxena S, Pant N, Jain DC, Bhakuni RS. Antimalarial agents from plant sources. Curr Sci. 2003;85(9):1314-29.
5. Verheij EWM, Coronel RE. Plant Resources of South-East Asia. Bogor Indonesia: Prosea. 1992.
6. Kochummen KM. Tree flora of malaya. A manual for foresters. Kuala Lumpur, Malaysia: Longman Malaysia Sdn. Berhard. 1973.
7. Hafid AF, Septiani RP, Fabriana LH, Febriyanti N, Ranggaditya D, Widyawaruyanti A. Antimalarial activity of crude extracts of artocarpus heterophyllus, artocarpus altilis, and artocarpus camansi. Asian J Pharm Clin Res. 2016;9(1):279-81.
8. Widyawaruyanti A, Subehan, Kalauni SK, Awale S, Nindatu M, Zaini NC, *et al.* New prenylated flavones from Artocarpus champeden, and their antimalarial activity *in vitro*. J Nat Med. 2007;61:410-3.
9. Hafid AF, Ariantari NP, Tumewu L, Hidayati AR, Widyawaruyanti A. The Active marker compound identification of *Artocarpus champeden* SPRENG. stembark extract, morachacone a as antimalarial. Int J Pharm Pharm Sci. 2012;4:246-9.
10. Trager W, Jensen JB. Reports: Human malaria parasites in countinous culture. Science. 1976;193:673-5.
11. Desjardins RE. *In Vitro* Techniques for Antimalarial Development and Evaluation. Peter W, Richards WHG., editor. Springer-Verlag (Germany): Handbook of Experimental Pharmacology. 1984.
12. Pink RJ, Hudson A, Mouries MA, Bendig M. Opportunities and challenges in antiparasitic drug discovery. Nat Rev Drug Discov. 2005;4(9):727-40.
13. Hardjono S, Bambang TW, Anindi LN, Widiandani T. Molecular docking of N-benzoyl-N′-(4-fluorophenyl) thiourea derivatives as anticancer drug candidate and their ADMET prediction. Research Journal of Pharmacy and Technology. 2019;12(5):2160.
14. Markham KR, Mabry TJ. Ultraviolet-visible and Proton Magnetic Resonance Spectroscopy of flavonoids. The Flavonoids. Boston, MA: Springer, 1975.
15. Qin X, Xing YF, Zhou Z, Yao Y. Dihydrochalcone Compounds Isolated from Crabapale Leaves Showed Anticancer Effects on Human Cancer Cell Lines. Molecules. 2015;20(12):21193-203.
16. Lotulung PD, Fajriah S, Hanafi M, Filaila E. Identification of cytotoxic compound from Artocarpus communis leaves against P-388 cells. J Biol Sci. 2008;11(21):2517-20.
17. Siems KJ, Mockenhaupt FP, Bienzle U, Gupta MP, Eich E. *In vitro* antiplasmodial activity of central American medicinal plants. Trop Med Int Health. 1999;4(9):611-5.
18. Portet B, Fabre N, Roumy V, Gornitzka H, Bourdy G, Chevalley S, *et al.* Activity-guided isolation of antiplasmodial dihydrochalcones and flavanones from Piper hostmannianum var. berbicense. Phytochemistry. 2007;68(9):1312-20.
19. Burger MCM, Fernande JB, Silva MFG, Escalante A, Prudhomme J, Roch KG, *et al.* Structures and Bioactivities of Dihydrochalcones from Metrodorea stipularis. J Nat Prod. 2014;77(11):2418-22.
20. Carroll AR, Fechner GA, Smith J, Guymer GP, Quinn RJ. Prenylated Dihydrochalcones from Boronia bipinnata that Inhibit the Malarial Parasite Enzyme Target Hemoglobinase II. J Nat Prod. 2008;71(8):1479-80.
21. Francis SE, Sullivan DJ, Goldberg DE. Hemoglobin metabolism in the malaria parasite Plasmodium falciparium. Annu Rev Microbiol. 1997;51:97-123.
22. Ettari R, Bova F, Zappala M, Grasso S and Micale N. Falcipain-2 inhibitors. Medicinal Research Reviews. 2009;30(1):136-67.
23. Pandey KC, Wang SX, Sijwali PS, Lau AL, McKerrow JH, Rosenthal PJ. The Plasmodium falciparum cysteine protease falcipain-2 captures its substrate, hemoglobin, via a unique motif. Proc Natl Acad Sci USA. 2005;102(26):9138-43.
24. Singh A, Kalamuddin MD, Mohmmed A, Malhotra P, Nasimul H. Quinoline-triazole hybrids inhibit falcipain-2 and arrest the development of Plasmodium falciparum at the trophozoite stage. RSC Adv. 2019;9:39410-21.
25. Korde R, Bhardwaj A, Singh R, Srivastava A, Chauhan VS, Bhatnagar RK, *et al.* Prodomain peptide of Plasmodium falciparum cysteine protease (falcipain-2) inhibits malaria parasite development. J Med. Chem. 2008;51(11):3116-23.

26. Bekono BD, Kang FN, Owono LCO, Megnassan E. Targeting Cysteine Proteases from Plasmodium falciparum: A General Overview, Rational Drug Design and Computational Approaches for Drug Discovery. Current Drug Targets. 2017;18(5).

27. Sijwali PS, Rosenthal PJ. Gene disruption confirms a critical role for the cysteine protease falcipain-2 in hemoglobin hydrolysis by Plasmodium falciparum. Proceedings of the National Academy of Sciences of the United States of America. 2004;101(13):4384-9.

GRAPHICAL ABSTRACT



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