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Original Article

Antimalarial Activity of Microalgae Extracts Based on Inhibition of PfMQO, a Mitochondrial *Plasmodium falciparum* Enzyme

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ABSTRACT

Malaria is an important global disease that threatened human life. The resistance *Plasmodium* sp. to the available medicines encourages the search for new antimalarial substances based on new mechanisms on the inhibition of PfMQO (the mitochondrial *Plasmodium falciparum* enzyme). **Objective:** The purposes of this study was to screen antimalarial substances from microalgae based on the inhibition of PfMQO. **Materials and Methods**: Five microalgae were extracted by maceration using chloroform pa and ethanol pa. These ten crude extracts obtained were tested for the inhibitory activity against the PfMQO enzyme. **Results:** The highest inhibitory activity against PfMQO enzyme was chloroform extract of *S. costatum* with 91.050% of inhibition and 0.043 µg/mL of IC_{50}. The ethanol extract of *S. platensis* showed 91.999% and 5.25 µg/mL of inhibition and IC_{50}, respectively. These results indicated that the two extracts provide high antimalarial activity exceeded a theoretical standard of antimalarial bioactive compounds. **Conclusion**: Chloroform extract of *S. costatum* and ethanol extract of *S. platensis* are promising sources of antimalarial compounds based on the inhibition of PfMQO.

Key words: Screening, Antimalarial, Microalgae, Inhibitory activity, *P. falciparum*.

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INTRODUCTION

Malaria is one of the world's health problems as almost half of the world's population is in the risk of malarial infection. This disease causes the mortality of 445,000 annually.[1] The disease is caused by the *Plasmodium* parasite infecting human erythrocytes through the bite of female *Anopheles* mosquito.[1] *Plasmodium falciparum* is the most dangerous species that can infect all erythrocyte stages, attacking all ages and sexes, including the most susceptible of pregnant women and children.[1] The incident of malaria is very high, causing severe complications to the patients, including shock, cerebral malaria, acute kidney failure, intravascular hemolysis, pulmonary edema and cause of deaths as the most.[2,3] The emergence of various side effects in patients and the resistance to *P. falciparum* parasites due to the use of available commercial drugs so far have encouraged the search for a new and more effective source of anti *P. falciparum* compounds. Based on the different mechanism of action, screening of antimalarial compound hopefully discover the new anti-*P. falciparum* compound without triggering the resistance and with a minor side effect.[4]

Microalgae contain various bioactive compounds with numerous biological activities that have been isolated and used for pharmaceutical industrial purposes.[5] Microalgae and cyanobacteria produce new bioactive compounds with various activities such as acetogenin, bromophenol, fatty acids, terpenes, sterols, alkaloids,[5,6] stanols, isoprenoids, terpenoids, steroid, phenolic compounds, acrylic acid and alkaloid[7], neopphytadiene and phytol[8], heptadecane and tetradecane.[9] The lipopeptide and various bioactive contents possess activities of cytotoxic (41%), antitumor (13%), antiviral (4%), antibiotics (12%), and the remaining 18% provide activities of antimalarial, antifungal, multi-drug resistance reversers, antifeedant and immunosuppressive agents, and also increase immunity and metabolism.[6] *Nostoc* sp. contains fukosianin that inhibits the growth of P. *falciparum* with IC_{50} of 8.4 µg/mL.[3] *Lyngbya aestuarii* Liebm provides antimalarial activity with IC_{50} of 18.18 µg / mL, whereas the *Oscillatoria baryana* also exhibits such activity with IC_{50} of 51 mg/mL against *P. falciparum* Pf3D7.[10]

MQO (Malate quinone oxidoreductase) is an enzyme that plays a role in the electron transport process of *P. falciparum* mitochondria. These enzymes are some of the keys to energy production. The *P. falciparum* MQO (PfMQO) are specific targets to evaluate the inhibitory activity of substances against *P. falciparum*. That is part of both mitochondrial ETC and TCA cycle, substituting other mitochondrial malate dehydrogenase.[11-15] This specific target minimizes the side effect because such pathways are not available in humans.[16] PfMQO catalyzes the oxidation of L-malate to oxaloacetate and simultaneously reduces the ubiquinone to ubiquinol. It is proven that this membrane protein is important for the survival of *P. falciparum* within the intra-erythrocytic asexual stage because it is involved in three pathways: ETC, tricarboxylic acid and fumarate cycle.[17-20] The inhibition of mitochondrial enzymes of *P. falciparum* without disturbing the function of human

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mitochondrial is a new promising action mechanism to overcome the resistance and negative side effects of medicines in patients.[4]

Up to now, the study of microalgae extracts from *Spirulina platensis*, *Chlorella vulgaris*, *Skeletonema costatum*, *Chaetoceros calcitrans* and *Nannochloropsis occulata* to inhibit the growth of *P. falciparum* by specific enzyme inhibiting mechanism of PfMQO have not been carried out yet. Therefore, this study was conducted to screen the antimalarial activity of the microalgae extracts as the sources of anti *P. falciparum* based on the inhibition of PfMQO.

MATERIALS AND METHODS

Microalgae samples

S. platensis, *C. vulgaris,* and *S. costatum* were cultivated at the Brackish Water Aquaculture Center in Jepara, Indonesia, while *C. calcitrans* and *N. occulata* were cultivated at the Brackish Water Aquaculture Center in Situbondo, Indonesia. Dried microalgae powders were obtained from both centers.

PfMQO enzymes

The PfMQO enzyme was provided by the Technology Assessment and Application Agency, Ministry of Research, Technology and Higher Education, Indonesia.

Extraction

Each microalgae powder of 310 g, was macerated in 700 ml chloroform pa, stirred at 800 rpm (30 minutes), and soaked for 24 hours. Then, the solution was filtered using the Buchner funnel to obtain the first filtrate. The retentates were macerated with chloroform pa as much as 500 ml, stirred at 800 rpm and left to stand for 24 hours to obtain the second filtrate. The two filtrates were combined and evaporated using a Rotary Vacuum Evaporator at a temperature of 50°C. Remaining retentates were subsequently macerated with 700 ml ethanol and stirred for 30 minutes at 800 rpm, and left to stand for 24 hours. After being filtered, the obtained ethanolic extract was evaporated and stored in refrigerator until used.[21]

Screening of inhibitors

Recombinant PfMQO used for screening several inhibitors and parasitic agents. The dried microalgae extracts were solubilized in methanol at a concentration of 0.02 mg/ml and sonicated for 5 minutes at 37°C by using a sonicator (AsOne sonicator, Japan). Due to the same principle employed by both assays, screening of PfMQO inhibitors adapted from the end-point assay method reported for Pf DHODH.[22,23] An assay mix composed of HEPES KOH pH 7.5, 20 µL 1 mM KCN, 8,3 µL 60 mM d-UQ (decylubiquinone), 200 µL 12 mM DCIP (blue) and 5.3 µL of PfMQO-membrane fraction was prepared. Next, 193 µl of assay mix was transferred to a 96-well plate containing 2 µl (10 µM) or 0.5 µl (2.5 µM) of 1 mM inhibitors (columns 2 to 11) or DMSO [column 1, negative control (0% inhibition)], mixed well and background recorded for 5 min at 600 nm and 37 °C using SpectraMax® Paradigm® Multi-Mode Microplate Reader (Molecular Devices). To start the reaction, five µl of 400 mM L-malate was added, mixed well and after 8 min the activity was recorded. The column 12 of 96-well plate was set as a positive control (100% inhibition), and the substrate was not added. The PfMQO inhibition was determined by end-point measurement at 8 min and analyzed by calculating the inhibition (%) relative to negative and positive controls, in duplo. The results were stated as active sample when the inhibitory activity value (IC_{50}) obtained was greater than 50%. The inhibition value was calculated based on the following formula:[4,24,25]

$$\%\ inhibition = 100 - \left[\left(\frac{sample - positive\ control}{negative\ control}\right) \times 100\ \%\right]$$

RESULTS AND DISCUSSIONS

Screening of antimalarial activity from microalgae

This paper describes the antimalarial activity of several microalgae based on the inhibitory activity against PfMQO enzyme. The results of the primary screening for antimalarial activities from ten crude extract showed that all of the crude extracts exhibited inhibition activity against PfMQO enzyme. In the first screening, we found that from a total of ten extracts, seven extracts (*S. platensis*, *C. vulgaris*, *S. costatum*, *C. calcitrans*, *N.occulata* chloroform extracts, and *S. platensis*, *S. costatum* ethanol extracts) exhibited high antimalarial activities. The ethanolic extract of *S. platensis* exhibited the highest antimalarial activity with inhibition activity of 91.99% at 320 µg/ml. The second and the third highest antimalarial activities were shown by the chloroform and ethanolic extracts of *S. costatum*. Chloroform and ethanol extracts of *S. costatum* showed 91.050% and 86.830% inhibition activities at 320µg/ml, respectively. High antimalarial activities were also shown by *C. calcitrans* chloroform extract with inhibition activity of 81.634% at 320 µg/ml, *S. platensis* chloroform extract with inhibition activity of 76.926%, *C. vulgaris* with inhibition activity of 76.844% and *N. occulata* chloroform extract with inhibition activity of 68.468% (Table 1).

A further experiment was conducted to evaluate dose-dependent activity of each extract. Generally, all extracts showed dose-dependent inhibiting-activity of PfMQO.

The all extracts showed dose-dependent inhibiting activity of PfMQO with coefficients correlation more than 0.9 (Figures 1-5) except chloroform extract of *S. costatum* (Figure 3). Based on the dose-dependent curve, the IC_{50} were calculated (Table 2).

The PfMQO enzyme plays a fundamental role in *P. falciparum* mitochondria, but this enzyme is not present in the mitochondrial human system, so the discovered drug based on this mechanism provide no any interference or side effects to the host.[4] This was a new mechanism on the target treatment of antimalarial compouds.[4] By inhibiting PfMQO enzyme, the mitochondrial electron transport chain (ETC) of *P. falciparum* does not occur. PfMQO catalyzed the oxidation of L-malate to oxaloacetate and the simultaneous reduction of ubiquinone to ubiquinol. This membrane protein was important for the survival of *P. falciparum* within the intra-erythrocytic asexual stage because it was involved in three pathways: ETC, tricarboxylic acid cycle and fumarate cycle.[17-19] The process of inhibition of the development of *P. falciparum* through inhibition of the energy transfer processes within the mitochondria without disturbing its host was a new mechanism

Table 1: Inhibition activity of various microalgae against *P. falciparum* PfMQO enzyme at 320 µg/ml.

Types of Microalgae	Inhibition Activity (%)	
	Chloroform	Ethanol
S. platensis	76.926	91.999
C. vulgaris	76.844	36.227
S. costatum	91.050	86.830
C. calcitrans	81.634	18.520
N. occulata	68.468	41.225

Table 2: IC_{50} microalgae extract against PfMQO enzymes of *P falciparum*.

Types of Microalgae	IC_{50} (µg/mL)	
	Chloroform	Ethanol
S. platensis	60.68	5.25
C. vulgaris	115.25	779.92
S. costatum	0.043	47.29
C. calcitrans	35.008	15476.64
N. occulata	128.067	689.227

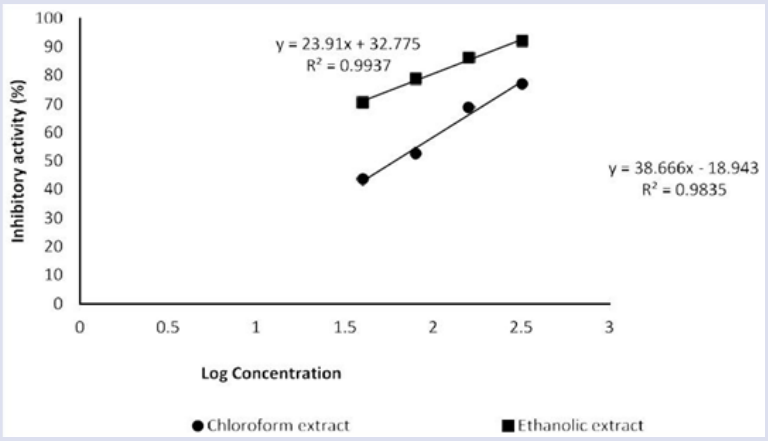

Figure1: PfMQO inhibiting-activity of *S. platensis* chloroform and ethanolic extracts.

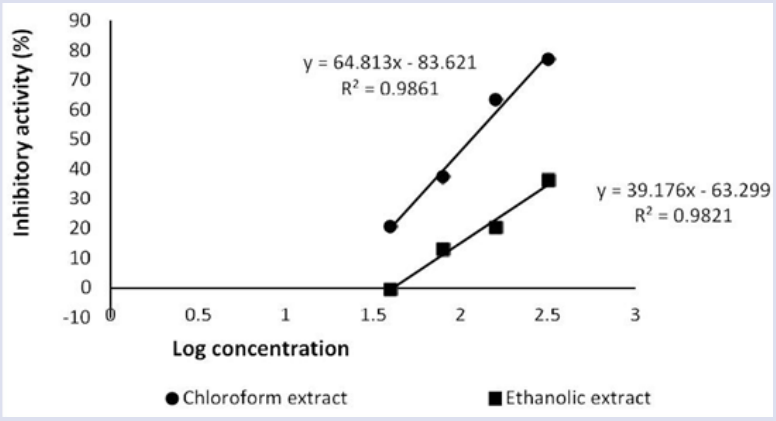

Figure 2: PfMQO inhibiting-activity of *C.vulgaris* chloroform and ethanolic extracts.

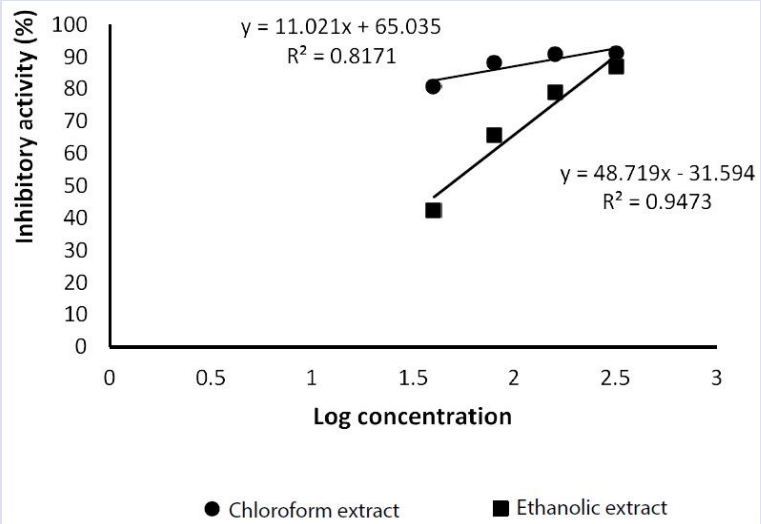

Figure 3: PfMQO inhibiting-activity of *S.costatum* chloroform and ethanolic extracts.

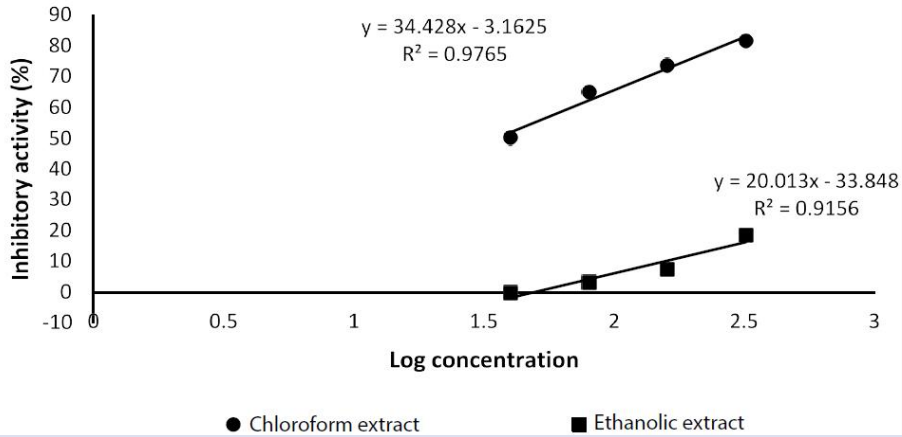

Figure 4: PfMQO inhibiting-activity of *C. calcitrans* chloroform and ethanolic extracts.

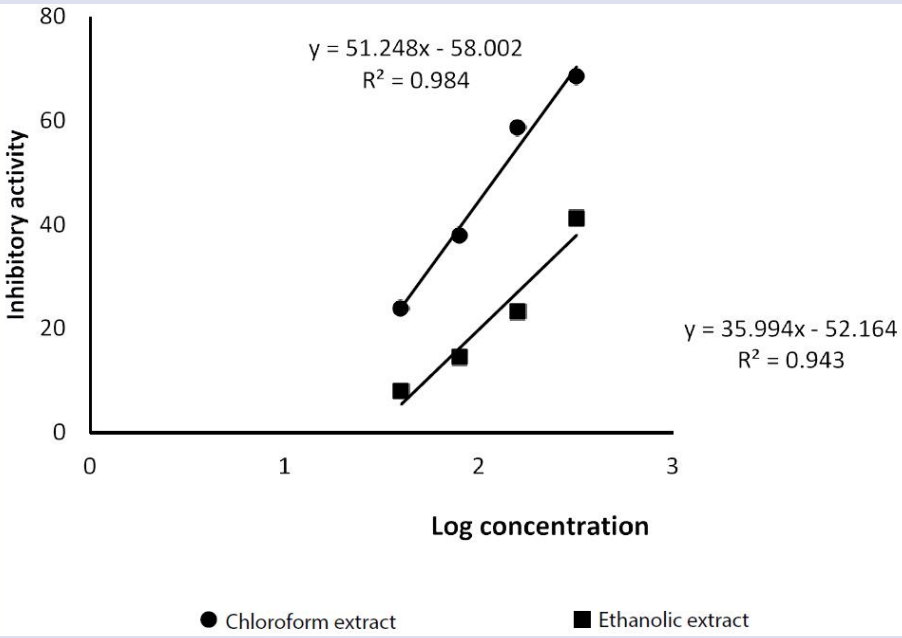

Figure 5: PfMQO inhibiting-activity of *N. occulata* chloroform and ethanolic extracts.

of action. Discovering new antimalarial agents based on the new mechanism hopefully overcome the drug resistance and negative effects in patients.[4]

IC_{50} of microalgae extracts against PfMQO

IC_{50} of chloroform extract and ethanolic extracts of *S. platensis* against PfMQO were 60.68 µg/mL and 5.25 µg/mL, respectively. The higher IC_{50} were found for C.*vulgaris* extracts either chloroform or ethanolic extracts with IC_{50} of 115.25 µ/mL and 779.92 µg/mL, respectively. Much higher activities were found in *S. costatum* either for chloroform or ethanolic extracts with IC_{50} of 0.043 µg/mL and 47.29 µg / mL, respectively. The IC_{50} value of *C. calcitrans* against the PfMQO enzyme was 35.008 µg/mL for the chloroform extract and 15,476.64 µg/mL for ethanol extract. The IC_{50} of *N. occulata* against PfMQO enzyme was 128.067 µg/mL for chloroform extract and 689.227 µg/mL for ethanol extract.

Bioactive compounds are categorized to be active and potent for antimalarials when the IC_{50} values of the compound less than 10 µg/mL, and to be moderate active when their IC_{50} values range from 10 µg/mL to 50 µg/mL, but will be categorized as inactive compound when IC_{50} values are more than 50 µg/mL.[26] The IC_{50} of chloroform extract of *S. costatum* and ethanolic of *S. platensis* extract was 0.043 and 5.25 µg/mL, respectively. This two extract might be categorized to the potent sources of antimalarial compounds. The activity might be higher when the compounds are purified. The other two extracts namely ethanolic extract of *S. costatum* and chloroform extract of *C. calcitrans* exhibited IC50 of 47.29 and 35.008 µg/mL, respectively implied that the extracts should be categorized to moderate active extracts. The other remaining extracts showed IC_{50} more than 50 µg/mL implying low activity or inactive extracts. The active extracts further should be tested directly against *P. falciparum* to confirm the inhibitory activity against PfMQO.

This work discovered that *S. costatum* and *S. platensis* were two promising sources of antimalarial compound for developing new antimalarial drugs. Further purification process should be carried out to obtain purified substance for chemical structure elucidation and further study on the bioactivity.

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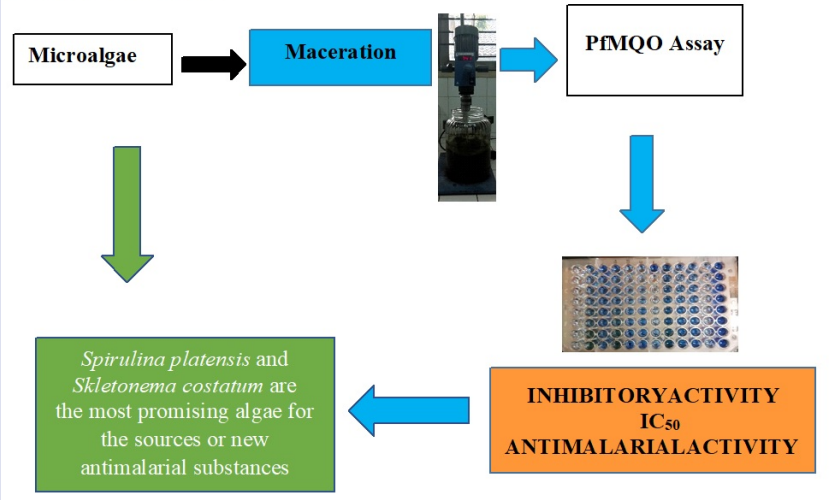
GRAPHICAL ABSTRACT



SUMMARY

- Seven samples from a total of ten microalgae extracts (chloroform and ethanol) exhibited high antimalarial activity.
- The highest antimalarial activity was shown by the ethanol extract of *S. platensis*.
- Antimalarial activity from *S.platensis* and *S. costatum* showed high inhibitory activity of *P. falciparum* and promising resources to developed antimalarial compounds.

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Google Scholar Link: https://scholar.google.co.id/citations?user=3fZJEFcAAAAJ&hl=en

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