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

Endang Ariyani Setyowati1,2, Alim Isnansetyo3,*, Tjut Sugandawaty Djohan1, Raden Wisnu Nurcahyo4, Erwahyuni Endang Prabandari5

**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 and ethanol. 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 IC₅₀. The ethanol extract of *S. platensis* showed 91.999% and 5.25 µg/mL of inhibition and IC₅₀ 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*.

**INTRODUCTION**

Malaria is one of the world’s health problems as almost half of the world’s population is in the risk of malaria 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*.² *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.³ 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.⁴ 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* compounds without triggering the resistance and with a minor side effect.⁵

Microalgae contain various bioactive compounds with numerous biological activities that have been isolated and used for pharmaceutical industrial purposes.⁶ Microalgae and cyanobacteria produce new bioactive compounds with various activities such as acetylgenin, bromophenol, fatty acids, terpenes, sterols, alkaloids,⁷ stanols, isoprenoids, terpenoids, steroid, phenolic compounds, acrylic acid and alkaloid,⁸ neophytdiine and phytol,⁹ heptadecane and tetradeacne.¹⁰ 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.¹¹ *Nostoc* sp. contains fukosianin that inhibits the growth of *P. falciparum* with IC₅₀ of 8.4 µg/mL.¹² *Lyngbya aestuarii* Liebm provides antimalarial activity with IC₅₀ of 18.18 µg/mL whereas the *Oscillatoria baryana* also exhibits such activity with IC₅₀ of 51 mg/mL against *P. falciparum* PBSD.¹³

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) is 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.¹⁴-¹⁵ 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.¹⁷-¹⁹ The inhibition of mitochondrial enzymes of *P. falciparum* without disturbing the function of human
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.

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 inhibited 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 91.05% and 86.83% inhibition activities at 320 µg/ml, respectively. High antimalarial activities were also shown by C. calcitrans chloroform extract with inhibition activity of 81.63% at 320 µg/ml, S. platensis chloroform extract with inhibition activity of 76.92%, C. vulgaris with inhibition activity of 76.84% 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 IC50 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 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.11-13 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>
<thead>
<tr>
<th>Types of Microalgae</th>
<th>Inhibition Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
</tr>
<tr>
<td>S. platensis</td>
<td>76.926</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>76.844</td>
</tr>
<tr>
<td>S. costatum</td>
<td>91.050</td>
</tr>
<tr>
<td>C. calcitrans</td>
<td>81.634</td>
</tr>
<tr>
<td>N. occulata</td>
<td>68.468</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Types of Microalgae</th>
<th>IC50 (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chloroform</td>
</tr>
<tr>
<td>S. platensis</td>
<td>60.68</td>
</tr>
<tr>
<td>C. vulgaris</td>
<td>115.25</td>
</tr>
<tr>
<td>S. costatum</td>
<td>0.043</td>
</tr>
<tr>
<td>C. calcitrans</td>
<td>35.008</td>
</tr>
<tr>
<td>N. occulata</td>
<td>128.067</td>
</tr>
</tbody>
</table>

Table 2: IC50 microalgae extract against PfMQO enzymes of P. falciparum.
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Figure 1: 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.
IC\textsubscript{50} of microalgae extracts against PfMQO

IC\textsubscript{50} of chloroform extract and ethanolic extracts of \textit{S. platensis} against PfMQO were 60.68 $\mu$g/mL and 5.25 $\mu$g/mL, respectively. The higher IC\textsubscript{50} were found for \textit{C. vulgaris} extracts either chloroform or ethanolic extracts with IC\textsubscript{50} of 115.25 $\mu$g/mL and 779.92 $\mu$g/mL, respectively. Much higher activities were found in \textit{S. costatum} either for chloroform or ethanolic extracts with IC\textsubscript{50} of 0.043 $\mu$g/mL and 47.29 $\mu$g / mL, respectively. The IC\textsubscript{50} value of \textit{C. calcitrans} against the PfMQO enzyme was 35.008 $\mu$g/mL for the chloroform extract and 15,476.64 $\mu$g/mL for ethanol extract. The IC\textsubscript{50} of \textit{N. occulata} against PfMQO enzyme was 128,067 $\mu$g/mL for chloroform extract and 689,227 $\mu$g/mL for ethanol extract.

Bioactive compounds are categorized to be active and potent for antimalarials when the IC\textsubscript{50} values of the compound less than 10 $\mu$g/mL, and to be moderate active when their IC\textsubscript{50} values range from 10 $\mu$g/mL to 50 $\mu$g/mL, but will be categorized as inactive compound when IC\textsubscript{50} values are more than 50 $\mu$g/mL.\textsuperscript{26} The IC\textsubscript{50} of chloroform extract of \textit{S. costatum} and ethanolic of \textit{S. platensis} extract was 0.043 and 5.25 $\mu$g/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 \textit{S. costatum} and chloroform extract of \textit{C. calcitrans} exhibited IC50 of 47.29 and 35.008 $\mu$g/mL, respectively implied that the extracts should be categorized to moderate active extracts. The other remaining extracts showed IC\textsubscript{50} more than 50 $\mu$g/mL implying low activity or inactive extracts. The active extracts further should be tested directly against \textit{P. falciparum} to confirm the inhibitory activity against PfMQO.

This work discovered that \textit{S. costatum} and \textit{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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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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Scopus Link: https://www.scopus.com/authid/detail.uri?authorId=6507970139
Google Scholar Link: https://scholar.google.co.id/citations?user=3fZJEFcAAAAJ&hl=en

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