Pharmacogn J. 2019; 11(5):929-935.
A Multifaceted Journal in the field of Natural Products and Pharmacognosy
www.phcogj.com

Original Article

Antiplasmodial Activity of Methanolic Leaf Extract of Mangrove Plants against *Plasmodium berghei*

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History

- Submission Date: 02-05-2019;
- Review completed: 30-05-2019;
- Accepted Date: 06-06-2019.

DOI : 10.5530/pj.2019.11.148

Article Available online

http://www.phcogj.com/v11/i5

ABSTRACT

Introduction: A mosquito-borne disease infected by *Plasmodium* is named as Malaria. Some drugs subjected to be active againts protozoans has developed resistance. It is very urgent to find alternative sources of new antimalarial agent. The main aim of this research was to study the activity of methanolic extracts of the leaf from mangrove plants on *Plasmodium berghei* by using *ex vivo* model. **Method:** Screening of antiplasmodial activity from methanolic leaf extracts of *Sonneratia alba*, *Acanthus ilicifolius* and *Sonneratia caseolaris* against *Plasmodium berghei* was carried out in this study. Antiplasmodial study was subjected *ex vivo* against *P. berghei* strain ANKA infected into Balb-C mice. Calculation of the percentage of parasitemia after 24 h observed in the model and a decrease in parasitemia level and inhibitory propagation were defined as the results. **Results:** Potential antiplasmodial activity shown by a decrease in parasitemia level and high inhibitory percentage was revealed by *S. alba* leaf methanolic extract at concentrations of 300, 100, 30, 10 and 3 µg/mL which provide the inhibition percentage of 95.5; 92,9; 78.7; 42.7 and 18.8%, respectively. Antiplasmodial activity can also be identified by the life cycle inhibition of plasmodium. Methanolic leaf extract of *S. alba* showed inhibition activity in the development of ring stage at minimum extract concentration of 300 µg/mL. At lower concentrations, trophozoites and schizones persisted with defects in morphological conditions. Moreover, Antiplasmodial activity of methanolic extracts of *S. alba* leaf was better than methanol extracts of *A. Ilicifolius* and *S. caseolaris* leaf. **Conclusion:** The results of this study indicated that among the mangrove plants have been studied, *S. alba* mangrove exhibited the highest antisplasmodial activity which moreover assumed as a potential source for natural antimalarial drug candidate.

Key words: *Sonneratia alba*, *Ex vivo*, *Plasmodium berghei*, Antimalaria, Mangrove.

Key Messages: The mangrove plant of *S. alba* was defined as a potential source of natural antimalarial drug candidate. The methanolic extract of *S. alba* leaf indicated that they contained phytochemical constituents and was proven scientifically to have antiplasmodial activity. The activity of methanolic extracts of *S. alba* leaf was better than that of of *A. Ilicifolius* and *S. caseolaris* leaf.

INTRODUCTION

Parasitic protozoa infection by the genus *Plasmodium* caused a serious health problem especially in tropical and subtropical countries called as Malaria disease.[1,2] 438,000 deaths with more than 214 million cases were noted in 2015 as reported by malarial disease.[2] At least, there are five species of Plasmodium causing malaria in human; *Plasmodium vivax, Plasmodium falciparum, Plasmodium ovale, Plasmodium malariae,* and *Plasmodium knowlesi*. One of the most virulent to be responsible for severe clinical malaria and death is *P. falciparum*.[1-3] Humans and female *Anopheles* mosquito as vectors were involved in *Plasmodium* life cycle. As the result, *P. berghei* infection in mice undergone similar life cycle in human was shown being studied in practical laboratory.[1]

Resistancy on antimalarial drugs has been developed by many protozoans, especially against *P. falciparum* causing disease severity of malaria. Long-term use of certain anti-malarial drugs in particular endemic area is the most responsible reason for this resistance. An approach to overcome malaria transmission is a need to respond *P. falciparum* resistance to antimalarial drugs such as chloroquine, quinine and amodiaquin[4-7] The increase of mortality by malaria disease was caused by the resistance to *P. falciparum*.[1-3,5] The reason for the development of new antimalarial drugs was also affected by the severity of infection and resistance of *P. Falciparum*.

Beneficial factor to affect research of plant utilization in Indonesia is high biodiversity which can be an additional element in medicinal research to produce new drug candidate exploration. A consideration to discuss the side effects of sythetics is more referred increase the use of natural source from plants for the treatment of many diseases. Several antimalarial compounds isolated from plants such as quinine and artemisinin were also provided. Within an effort of exploration on new natural medicine as antimalaria, study on various types of plants in Jambi Province having different geographical conditions which traditionally has been used by the community as

Cite this article: Muhaimin M, Latief M, Putri RD, Chaerunisaa AY, Aditama AY, Pravitasari NE, *et al.* Antiplasmodial Activity of Methanolic Leaf Extract of Mangrove Plants against *Plasmodium berghei*. Pharmacog J. 2019;11(5):929-35.

traditional medicine for the treatment of malaria has been conducted.

The seashore is most productive and biologically important ecosystems in the world which provide important and unique ecosystem and coastal marine systems. Breeding and nursing land for marine and pelagic species were further provided as a source of food, medicine, fuel and building materials for local communities.[5,8-10] One of the forest widely available in this area is Mangrove. Part of the interface between land and sea in Jambi which located in the east coast of Sumatra occupied by Mangrove Forest. In this case, Mangrove plants provides potency of traditional medicine to treat several diseases.[8,11,12] Being located at the interface between land and sea, morphological and physiological adaptability of mangrove plants to extreme conditions has been developed by having high adaptability to deal with natural stress. Mangroves are also known to have biochemically habitats with a variety of unique species to provide new natural products which potential as a source of substances for medicinal treatments.[13] An early indicator of potential antimalarial activity can be used is antiplasmodial. These study was conducted to explore the scientific prove of mangrove plants having antoplasmodial activity which potent to be developed as new natural anti malarial agent.

MATERIALS AND METHODS

Plant material

The collection of *Sonneratia alba* (Sonneratiaceae), *Acanthus ilicifolius* (Acanthaceae) and *Sonneratia caseolaris* (Sonneratiaceae) leaves were accomplished in January 2017 from a Mangrove forest in Muara Sabak (Tanjung Jabung Timur, Jambi) and identified by a taxonomist from Department of Biology, Faculty of Science and Technology, University of Jambi.

Chemicals and reagents

In the current study, analytical grade was applied by all chemicals. The chemicals purchased were potassium peroxodisulfate, Quercetin dehydrate, gallic acid, anhydrous sodium carbonate (Na_2CO_3), aluminum tri chloride, potassium acetate, sodium acetate, ferric chloride hexahydrate ($FeCl_3.6H_2O$), Folin–Ciocalteu reagent, Dragendorff's reagent, mercuric chloride, potassium iodide, iodine were purchased from Sigma–Aldrich. Ethanol, methanol, hydrochloric acid (HCl), sulfuric acid (H_2SO_4), chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH) were purchased from Merck. Those chemicals and reagents were used without further purification.

Extraction

As chronological steps to make the extract covered 2 kg of dried leaves each mangrove (*S. alba, A. ilicifolius* and *S. caseolaris*). By maceration techniques at room temperature, they were grinded and extracted with 2.5 L of methanol, 3 times, (24 h each). A rotary evaporator (buchi rotavapor R-205) concentrated and evaporated the filtrate in a vacuum at 60°C. As the result, there was rendement value (1.8% w/w) of extract which been stored as the dried extract in the refrigerator at 4°C for further used.[9,14,15]

Phytochemical screening

In accordance with qualitative phytochemical screening tests, the researchers carried out Phytochemical screening of the methanolic leaf extract.[3,12] The major aim of the study focused to evaluate the presence of chemical constituents such as alkaloids, flavonoids, saponins, triterpenoids, steroids, tannins, glycosides and phenolics.

Plasmodium and animal

P. berghei from Eijkman Institute for Molecular Biology, Jakarta, was used in plasmodium test in this study. Furthermore, mice of BALB/c species was also used as the animal to perform *Ex vivo* study of antiplasmodial activity. They were cultivated in Eijkman Institute for Molecular Biology, Jakarta.

Ex vivo erythrocythic-antiplasmodial assay

Ex vivo study means that which takes place outside an organism. In science, *ex vivo* refers to experimentation or measurements done in or on cells or tissues in an artificial environment outside the organism (cultured in a laboratory apparatus e.q 96 well plates) with the minimum alteration of natural conditions. *Ex vivo* conditions allow experimentation under more controlled conditions. Concentrations of 300, 100, 10 and 3 µg/mL for *Ex vivo* antiplasmodial activity of the methanolic leaf extract of mangrove plants (*S. alba*, *A. ilicifolius* and *S. caseolaris*) were used. The result were compared with that from control groups treated with distilled water (containing dimethylsulfoxide 10%, as solvent of extracts). The reference groups were treated with standard drugs (pyremethamine). The i.p. administration of donor ICR mice blood contain about 1×10^6 parasites induced Malaria infection in mice. *Ex vivo* study against *P. berghei* strain ANKA were conducted by testing the extracts into infected Balb-C mice blood. The preparation of blood smears collected from tail of each mice at 24 h. Parasitized erythrocytes of the blood sample films were determined by staining with Giemsa's stain. Three times of the treatments were conducted as replications. The results were observed as inhibition of parasitemia in mice blood cells. Decreased levels of parasitaemia and percent of inhibition was expressed as antiplasmodial activity observed by calculating the parasitaemia percentage after 24 h observation.

RESULTS

Phytochemical screening

The presence of secondary metabolites such as saponins, tanins, phenolics, glycosides, alkaloids, steroids, flavonoids, and terpenoids, as presented in Table 1 was noted by the phytochemical screening of methanolic leaf extract of Mangrove plants (*S. alba*, *A. ilicifolius* and *S. caseolaris*). More spesifically, medicinal activity was recognized and detected by the phytochemical compounds.

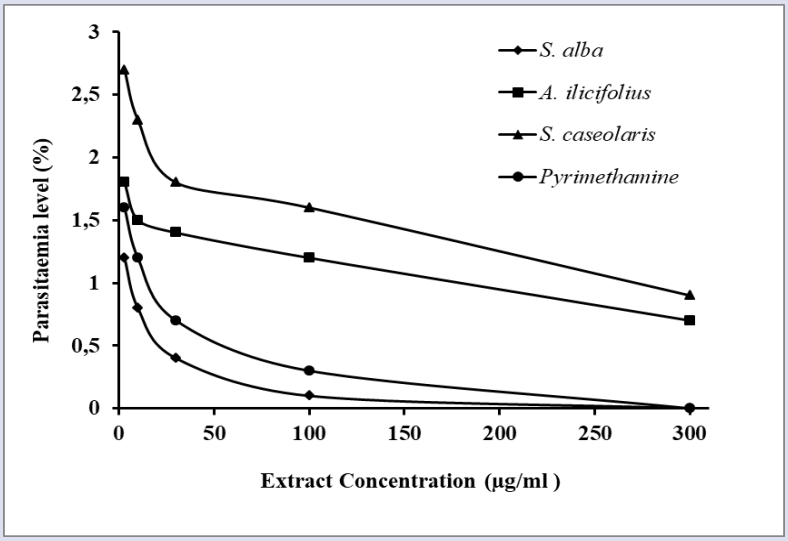
Ex vivo erythrocythic-antiplasmodial assay

It needs to emphasize that decreased parasitaemia levels or percentage of suppression expressed the antiplasmodial activity. As seen in Figure 1a, the parasitaemia level might be reduced with treatment of infected mice blood with 3, 10, 30, 100 and 300 µg/mL methanolic extract of *S. alba*, *A. Ilicifolius* and *S. caseolaris* leaf (Figure 1a). As the evidence, the methanolic extract of *S. alba*, *A. Ilicifolius* and *S. caseolaris* leaf suppresed the amount of parasitaemia caused by *P. berghei*. The level of parasitemia suppression by methanolic extract of *S. alba* leaf

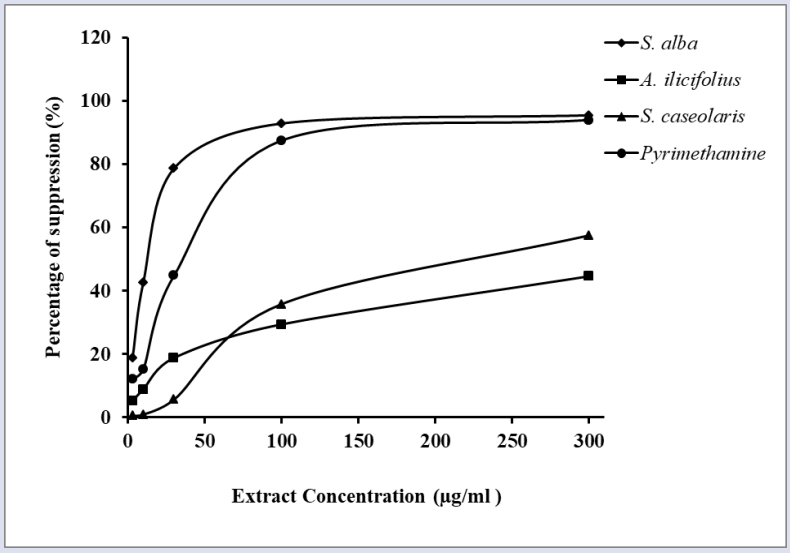
Table 1. Phytochemical screening of methanolic leaf extract of *Mangrove plants*.

Test	Methanolic leaf extracts of Mangrove Plants		
	Sonneratia alba	Acanthus ilicifolius	Sonneratia caseolaris
Alkaloid	+	+	+
Flavonoids	++	++	++
Terpenoids	+	+	-
Phenolic	+	+	+
Steroids	-	-	+
Tanins	+	+	+
Saponins	+	+	-
Quinones	+++	-	+
Glycosides	+	+	+

+ = presence, – = absence



(a)



(b)

Figure 1: Erythrocythic-antiplasmodial assay of the three methanolic extracts of mangrove plants: *Sonneratia alba* (*S. alba*), *Acanthus ilicifolius* (*A. ilicifolius*), *Sonneratia caseolaris* (*S. caseolaris*) and pyrimethamine as positive control at established *P. berghei* infection (after incubated 24 h) observed as (a) Parasitaemia level and (b) Percentage of inhibition.

showed better activity than that of pyremethamine as standard drug. The data revealed that the suppression of parasitaemia percentage of infected erythrocythic by the methanolic extract of *S. alba* were observed at dose of 30, 100 and 300 µg/mL as presented in Figure 1b. Methanolic leaf extract of *S. alba* showed percentage of 95.5; 92.9; 78.7; 42.7 and 18.8% at concentrations of 300, 100, 30, 10 and 3 µg/mL, respectively as inhibition or percentage of suppression (Figure 1b). In conclusion, suppression of three methanolic leaf extracts of mangrove plants emphasized on decreasing parasitaemia levels and increase on percentage of inhibition of plasmodium. Hence, it can be inferred that methanolic extract of *S. alba* leaf posessed antiplasmodial activity to be developed as potent drug candidate for malaria. It can also be stated that the most potential antimalarial drug candidate is *S. alba* leaf, since it showed the highest activity among the three mangrove plants.

An additional way to observe antiplasmodial activity is by observing the inhibition of plasmodium life cycle. Schizonts, the development ring stage of Plasmodium life cycle, was also inhibited by Methanolic leaf extract of *S. alba* antiplasmodial activity in which it was not found at 300 µg/mL of extract treatment as presented in Figure 2e. Moreover, trophozoite and schizonts stage still could be detected with morphological defect conditions showing that at lower concentration they could survive from extract treatment (Figures 2a-d). Overall, since the ring stage development in plasmodium life cycle was also inhibited by mangrove plant *S. Alba, it* was then identified as a potential source of natural antimalarial drugs.

DISCUSSION

As mentioned previously, a major parasitic infectious disease in many tropical and subtropical regions named 'Malaria' has been increasing since the emergence of drug-resistant *Plasmodium falciparum*. As many as 207 million people suffered from malaria, with up to 627,000 deaths each year was reported by the World Health Organization (WHO). As a major killer, drug-resistant *P. falciparum* malaria, becomes one of the most difficult obstacles to combat. Therefore, an urgent matter

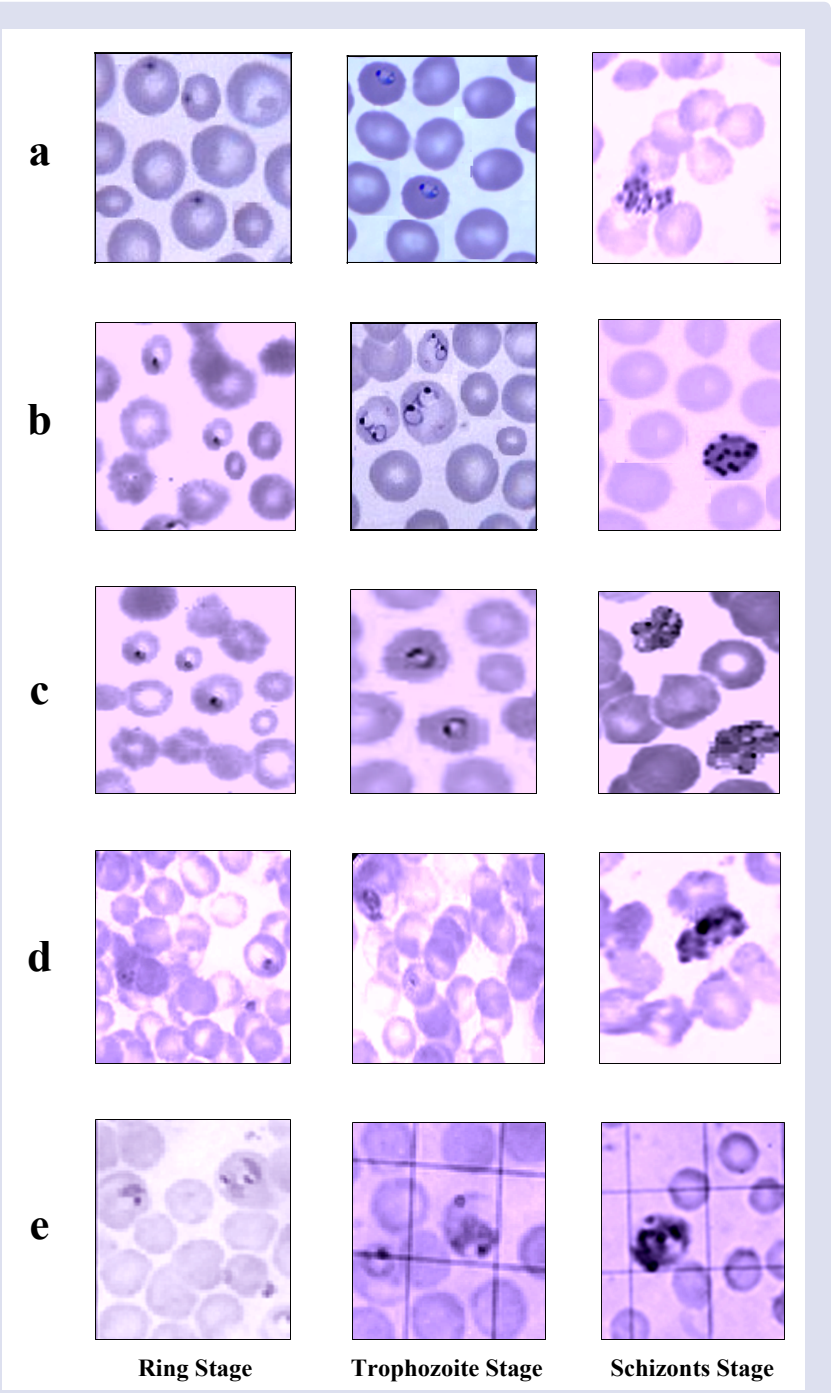


Figure 2: Effect of methanol extract of *S. alba* leaf on the life cycle of plasmodium at concentrations of (a) 3 µg/mL, (b) 10 µg/mL, (c) 30 µg/mL, (d) 100 µg/mL and (e) 300 µg/mL. (1000X Magnification).

is to develop a new class drug, and a useful way for screening and discovering plant extract with antiplasmodial activity.[1,3,16,17]

It is useful to explore new antimalarial therapy through biodiversity and traditional medicine knowledge, as it was in the case of artemisin.[17-19] Muhaimin (2018), for instance, carried out a study focused on mangrove plants located in tropical Jambi forest, traditionally utilized by people in various rural area to cure some diseases, in particlar malaria.[10] Potent *in vivo* antiplasmodial activity of the aerial parts and root crude extracts of these plant specieses was revealed from former study.[8,10,12] Antimicrobial activities and strong antioxidant activity was indicated and exhibited by the leaf of *S. alba, A. Ilicifolius* and *S. caseolaris*.[12,13] An investigation on antiplasmodial activity of methanolic leaf extracts of *S. alba*, *A. Ilicifolius* and *S. Caseolaris* against *Plasmodium berghei* reported in this study was confirmed with regard to its use as malarial remedy in Jambi ethnomedicine. The data of phytochemical screening presented in Table 1 found that methanolic extract of *S. alba*, *A. Ilicifolius* and *S. caseolaris* leaf contained saponins, glycosides, quinones, tanins, alkaloids, terpenoids, phenolic, and flavonoids. Antiplasmodial activity was reported to have connection with secondary metabolites of the plants such as alkaloids, flavonoids, quinones and triterpenoids.[5,8,12]

The data showed that Antiplasmodial activity from methanolic extracts of *S. alba* leaf is better than that of *A. ilicifolius* and *S. caseolaris* leaf.

This indicated that protozoas has been modulated by the extract. The accumulation of phytochemical constituents is a factor to affect the potential *S. alba* that may exert antimalarial activity either by causing elevation of red blood cells oxidation or by inhibiting protein synthesis.[11-13] The popularity and wide usage of this plant in folk medicine practices was used as evidence to support the main data of the study.

The antiplasmodial activity of *S. alba* leaf was almost similar with that from Pyrimethamine as presented in Figure 1a and 1b. Phytochemical constituents in the leaf is responsible for the observed antiplasmodial activity. To promote schizonticidal activity, potential antioxidant of some plants and natural products especially quinones have been occured by modulating the cellular signalling pathway.[11-13] Some study reported that antiplasmodial activity of compounds such as kempferol-3-O-rhamnoside, quercetin, methyl gallate and gallic acid, is a responsible as elevated free radicals levels commonly featured in malaria disease implicated in severe malaria complications. Further, phenolics and quinones with antioxidant activity were contained in extract as one of the modes of action of its extract.[12,13,16] Study on mechanism of Quinones as antimalarial revealed that it exert antiplasmodial activity by chelating with nucleic acid base pairing of the parasite.[19,20] With regard to plasmodicidal activity of ethanolic leaf extract of *S. alba* it could be stated this activity is contributed to quinone present in this plant and therefore the mechanism of antiplasmodial effect of the extract can be explained.

From the decreasing parasitaemia levels and increasing percentage of inhibition as presented in Figures 1a and 1b, it can be inferred that antimalarial activity has been possessed by the methanolic of *S. alba* leaf. Phytochemical constituents contained in the extract has proven scientifically to be responsible on the antimalarial activity. An integral part of medicinal plants is constituted by Phytochemicals and has responsible for their numerous bioactivities. Antiplasmodial activities of numerous plants containing a wide variety of phytochemicals as their bioactive principle has been extensively studied[10,12,16] Although there is no evaluation on the mechanism of the action of the leaf extract in the present study, antiplasmodial activities however can be explained by bioactive compounds detected from phytochemical screening such as alkaloids, flavonoids, and terpenoids.

Besides, antiplasmodial activity of the extract was also proved by the inhibition of plasmodium life cycle at concentration of 300 µg/mL (Figures 2a-d). Methanolic leaf extract of *S. alba* inhibited the development of ring stage called as schizonts which can be observed as cell death at concentration above 300 µg/mL (Figure 2e). Trophozoite and schizonts still survived with defect morphological conditions at concentration lower than 300 µg/mL (Figures 2a-d).

These results indicated that there was a potent antisplasmodial activity from mangrove plant *S. alba* which can be developed as new source of natural antimalarial drugs. Somehow, the effect of each constituent on antiplasmodial and antimalarial activity needs to be studied by further research.

CONCLUSION

In line with the decreasing parasitaemia levels and percentage of inhibition, an antiplasmodial activity was belonged to methanolic extracts of *S. alba* leaf. Hence, this indicated that mangrove plant of *S. alba* was defined as a potential source of natural antimalarial drug candidate. Methanolic extracts of *S. alba* leaf showed Antiplasmodial activity better than that of *A. Ilicifoliu*s and *S. caseolaris* extract.

ACKNOWLEDGEMENT

The research work was funded by a grant from Research Grant of National Innovation System with scheme Research Collaboration on 2017, Ministry of Research, Technology and Higher Education Indonesia.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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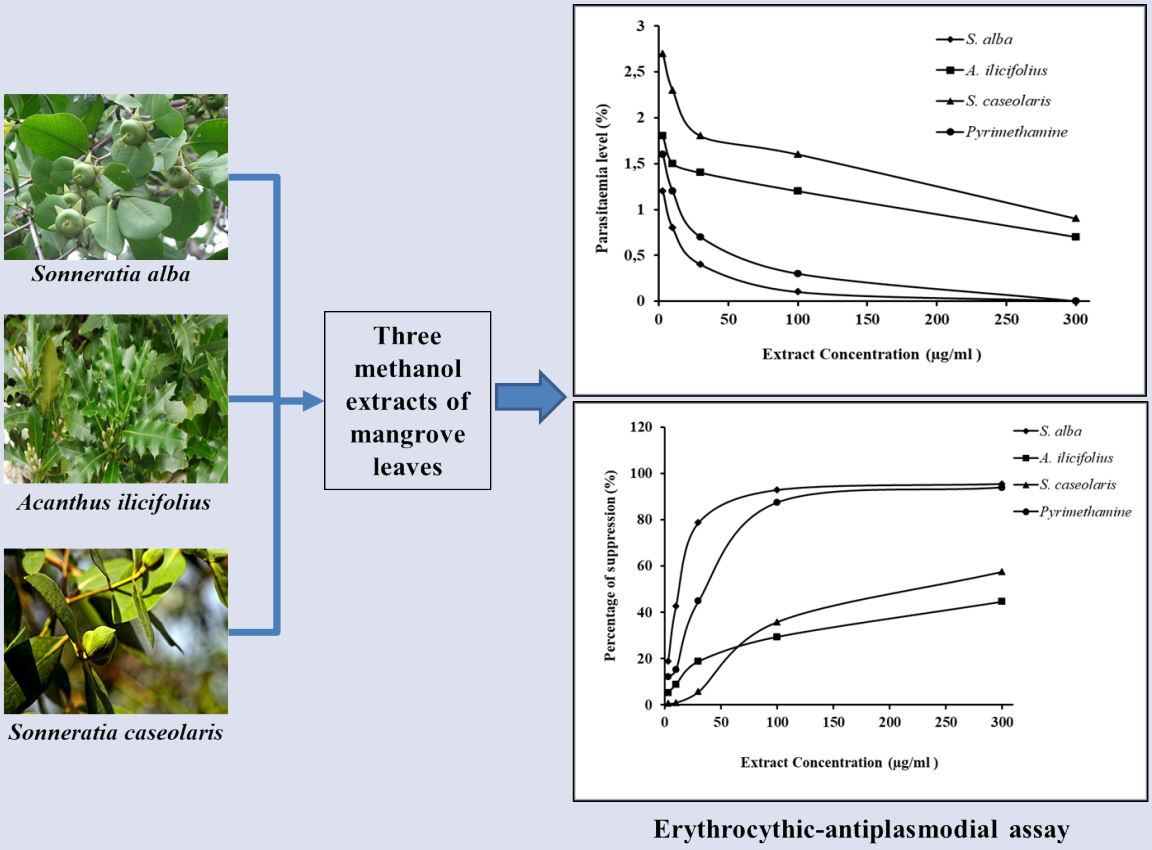
GRAPHICAL ABSTRACT



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Normalita Eka Pravitasari: Junior Researcher at Eijkman Institute, Jakarta, Indonesia. She has an interest in the malarial disease and infectious disease.

Josephine Elizabeth Siregar: Senior Researcher at Eijkman Institute, Jakarta, Indonesia. She has been interested in the relationship between the mitochondrial function and infectious disease, in particular the potential of mitochondria of the malaria parasite as drug target. The malaria parasite has a complex life cycle involving sudden changes of environment during transmission between different hosts and transition from extra- to intracellular existences, requiring specific adaptive differentiation. Despite the obvious importance of the energy transducing machinery in such adaptive process – which involves aerobic, semi aerobic and relatively anaerobic environment –remarkably little is known about the biogenesis of the mitochondrial energy transducing membrane in the malaria parasite. By acquiring detail knowledge of the biogenesis and function of the parasite's energy transducing membrane, and particularly the role of the parasite's two extrachromosomal DNAs in this process, it is hoped that new therapeutic targets for malaria treatment and prophylaxis could be identified.

Cite this article: Muhaimin M, Latief M, Putri RD, Chaerunisaa AY, Aditama AY, Pravitasari NE, *et al*. Antiplasmodial Activity of Methanolic Leaf Extract of Mangrove Plants against *Plasmodium berghei*. Pharmacog J. 2019;11(5):929-35.