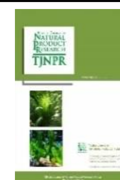
Trop J Nat Prod Res, April 2024; 8(4):6884-6890 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article



Antiplasmodial Activity of Ethanol Extract of *Sonneratia alba* Leaves

Muhaimin Muhaimin[1,4,*], Nor Latifah[1,2], Anis Y. Chaerunisaa[1], Anas Subarnas[1], Yasmiwar Susilawati[1,4], Rifanza Hirzan[3]

[1]*Faculty of Pharmacy, Universitas Padjadjaran, Jl. Raya Bandung - Sumedang Km 21, Jatinangor 45363, Indonesia*
[2]*Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, Jalan S. Parman, Banjarmasin, 70123, Kalimantan Selatan, Indonesia*
[3]*Faculty of Medicine, Universitas Padjadjaran, Jl. Raya Bandung - Sumedang Km 21, Jatinangor 45363, Indonesia*
[4]*Center of Herbal Study, Universitas Padjadjaran, Jl. Raya Bandung - Sumedang Km 21, Jatinangor 45363, Indonesia*

ARTICLE INFO

Article history:
Received 27 January 2024
Revised 31 March 2024
Accepted 06 April 2024
Published online 01 May 2024

Copyright: © 2024 Muhaimin *et al*. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Sonneratia alba (*S. alba*) is a mangrove plant that represents a promising natural source for potential antimalarial drug development. This study investigates the antimalarial effects of the ethanol extract derived from *S. alba* leaves in a mouse model. To assess the antimalarial activity of the extracts *in vivo*, mice were intraperitoneally injected with the *Plasmodium berghei* ANKA strain, followed by daily administration of the ethanol extract from *S. alba* leaves for four consecutive days. Parasitemia levels were monitored using light microscopy. The findings revealed a significant suppression of parasitemia in malaria-infected mice treated with the ethanol extract of *S. alba* leaves at a dose of 300 mg/kg BW, achieving a reduction of 77.34%. Mice administered with 150 mg/kg BW of the ethanol extract demonstrated a suppression rate of 62.35%, while those treated with 75 mg/kg BW displayed 43.53% suppression. This study underscores the potential antimalarial properties of the ethanol extract derived from *S. alba* leaves when administered *in vivo*.

Keywords: Antiplasmodial, Antimalarial, *Sonneratia alba*, *Plasmodium berghei*, Parasitemia.

Introduction

Malaria, a disease triggered by parasitic organisms belonging to the *Plasmodium* genus and spread through bites of female *Anopheles* mosquitoes, continues to pose a substantial health threat on a worldwide scale, especially in tropical and subtropical zones. In 2015 alone, approximately 214 million cases were reported, resulting in 438,000 deaths. The escalation of resistance to traditional medications presents a grave global challenge. Contemporary treatments for uncomplicated falciparum malaria commonly include artemisinin-based combination therapies (ACTs), which consist of a rapid-acting artemisinin derivative paired with a slower-acting adjunct medication.[1,2] Despite being extensively utilized, the effectiveness of ACTs has been hindered by delayed clearance of parasites, resulting in treatment failures.[1–6] In 2019, the World Health Organization (WHO) estimated 229 million new cases of malaria and 409,000 deaths, emphasizing the pressing necessity for alternative, improved antimalarial interventions.[1,2,6,7–14]
With vaccination efforts proving unsuccessful and resistance to existing drugs on the rise, researchers are actively seeking novel therapeutic options. In addition, *Plasmodium falciparum* is identified as the deadliest among the five species of *Plasmodium* responsible for human malaria, which also include *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi*. This particular species is responsible for the majority of fatalities associated with malaria.[1,2,6,13–20]
Enhancing malaria prevention and control efforts is imperative, given the escalating challenges posed by drug resistance and the gravity of the disease, particularly in cases caused by *Plasmodium falciparum*.

*Corresponding author. E mail: muhaimin@unpad.ac.id
Tel: +62 081394014688

Citation: Muhaimin M, Latifah N, Chaerunisaa AY, Subarnas A, Susilawati Y, Hirzan R. Antiplasmodial Activity of Ethanol Extract of *Sonneratia alba* Leaves. Trop J Nat Prod Res. 2024; 8(4):6884-6890. https://doi.org/10.26538/tjnpr/v8i4.19

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Overcoming *P. falciparum* resistance to key antimalarial drugs like chloroquine, quinines, and amodiaquine is crucial to curtailing malaria transmission. Resistance typically emerges following prolonged use of specific antimalarials within endemic regions, exacerbating malaria-related mortality rates.[1,2,6,21–28]
The urgency to address infection severity and resistance in *P. falciparum* has prompted the development of novel antimalarial agents. Leveraging Indonesia's rich biodiversity for scientific inquiry and medicinal plant exploration holds significant promise. Numerous antimalarial compounds, including quinines and artemisinin, have been sourced from botanicals. Harnessing plant-derived antimalarials presents a viable strategy for innovation, offering potential solutions to combat parasite resistance to existing drugs.[1,2,29–34]
Numerous pharmacotherapeutics originate from natural sources, serving as foundational compounds for the synthesis of antimalarial medications.[35–40] Quinine, extracted from the bark of the cinchona tree, has been a longstanding treatment for malaria.[2,35,36,37–41] This natural compound has served as a precursor for the development of more potent derivatives, such as chloroquine and primaquine.[2,6,42–48] Similarly, artemisinin, isolated from *Artemisia annua* by Chinese researchers, has yielded a family of antimalarials including dihydroartemisinin, artemether, and artesunate. These derivatives exhibit enhanced efficacy against malaria parasites.[2,14,27,34,49–52] The success of antimalarial drugs derived from natural plants underscores the potential of botanical ingredients for further pharmaceutical exploration.[1,2,6,48,50–52] Consequently, plant materials continue to inspire the development of novel antimalarial therapeutics.
"*Perepat*," interchangeably referred to as *Sonneratia alba*, denotes a species of mangrove plant that exhibits a widespread distribution, primarily thriving in temperate and tropical zones.[6,48] The leaves of *S. alba*, known for their resilience, boast a myriad of therapeutic properties including anti-inflammatory, antioxidant, antispasmodic, antiparasitic, antipyretic, antibacterial, detoxifying, wound-healing, and antimicrobial attributes. Remarkably, the unrefined ethanol extract derived from *S. alba* leaves demonstrated remarkable effectiveness against malaria, possibly due to the existence of secondary bioactive metabolites in the plant foliage. To confirm these results, the current research utilized a mouse model to authenticate the antimalarial potency of the aforementioned extracts.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

Trop J Nat Prod Res, April 2024; 8(4):6884-6890 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Materials and Methods

Plant sample collection and Identification

Fresh *S. alba* leaves were collected in the month of March 9, 2019 from a Mangrove forest in Muara Sabak (Tanjung Jabung Timur, Jambi) and identified by a taxonomist from Padjadjaran University's Department of Biology, Faculty of Mathematics and Natural Sciences, where a voucher specimen (No. 036/HB/05/2019) was deposited. To eliminate dirt and soil, the fresh leaves of *S. alba* were thoroughly washed. After that, the leaves were placed in the dry room. After drying, they were ground into small particles and stored in a sealed container at room temperature until further usage. The samples were rinsed with tap water and dried for three days at 60°C in a hot air oven (Memmert, Model; SFE600, Germany) before being powdered with a herb grinder (Jincheng, Model; SF, China).

Extraction procedure

A total of 3 kg of dried *S. alba* leaves were utilized for the extraction process sequentially. The leaves were either ground or chopped into smaller pieces to facilitate optimal and efficient extraction, leveraging the increased surface area for enhanced extraction kinetics. Extraction was performed three times using 30 L of ethanol through maceration techniques at room temperature (24 hours per cycle). Ethanol was chosen as the solvent due to its safety, non-toxic nature, and ability to dissolve phytochemical compounds effectively, thereby facilitating the extraction process by attracting these compounds. Employing multiple extraction cycles ensured maximal extraction of secondary metabolites. Ethanol was selected as the solvent owing to its polarity, enabling the filtration or extraction of polar, semi-polar, and non-polar compounds. Additionally, ethanol is non-toxic and miscible with water, further contributing to its suitability for this purpose. Following extraction, the macerate underwent concentration, evaporation, and drying under reduced pressure at a temperature of 60°C utilizing a rotary evaporator (Buchi Rotavapor R-205). The resulting output was recorded at 16.5% (w/w). The extract was preserved at a temperature of 4°C in a refrigerated storage unit until required for further analysis or experimentation.

Phytochemical screening

Standard protocols were employed to conduct qualitative phytochemical screening of the ethanol leaf extract, identifying the predominant classes of compounds present, including saponins, flavonoids, phenols, glycosides, steroids, terpenoids, tannins, and alkaloids.[4,6,48]

Plasmodium and animal models

The *Plasmodium* test utilized the *P. berghei* ANKA strain acquired from the Eijkman Institute for Molecular Biology in Jakarta. BALB/c mice (20–30 g; 6 to 8 weeks old) sourced from the Center for Life Sciences, Bandung Institute of Technology, served as the *in vivo* test subjects for antiplasmodial activity assessment. Approval for all experimental procedures concerning animals was granted by the Ethical Committee of Padjadjaran University (Approval No. 1244/UN6.KEP/EC/2019). The animals were housed in a controlled environment with adequate climate conditions, were provided *ad libitum* access to food, and underwent a minimum one-week acclimatization period before the commencement of the study.

Four-day suppressive test

Schizonticidal activity was assessed using Peters' four-day suppression test technique.[3] Mice were randomly assigned to six groups, each comprising five individuals. Group I acted as a negative control, receiving a solution containing 1% Pulvis Gummi Arabicum (PGA) and 3% ethanol in distilled water, that functioned as the carrier for the crude extracts and medication. Group II, which served as a negative control, got a comparable PGA and ethanol solution in distilled water. Group III acted as the positive control, receiving 36.4 mg/kg BW artesunate. Groups IV, V, and VI were treated with an ethanol extract of *S. alba* leaves at dosages of 75, 150, and 300 mg/kg BW, respectively. The extracts were given orally to each group by gavage. Mice were injected intraperitoneally with 1×10^6 *P. berghei*-infected red blood cells to cause malaria infection. The initiation of test extract administration began three hours after infection (referred to as D0) and persisted daily for three consecutive days (at 24-hour intervals post-infection: 24-, 48-, and 72-hours). Blood samples were collected from the tail vein on the fourth day to prepare thin films. Subsequently, the films underwent staining with a 10% Giemsa solution and were observed under a light microscope (Olympus, Model: CX-31, Japan). The count of infected red blood cells (iRBCs) was conducted in three distinct fields, each encompassing approximately 300 red blood cells. The mean of these counts was employed to calculate the percentage of parasitemia using the formula outlined below.

$$\%parasitemia = \frac{the\ number\ of\ infected\ red\ blood\ cells}{the\ number\ of\ total\ red\ blood\ cells} \times 100 \quad (1)$$

The level of suppression of parasitemia was determined using the formula provided below.

$$\%suppression = \frac{[A-B]}{A} \times 100 \quad (2)$$

In the equation, *A* signifies the mean percentage of parasitemia detected in the negative control group, whereas *B* represents the mean percentage of parasitemia observed in the group receiving treatment with the extract.

Statistical analysis

The experimental findings, collected from three independent replicates, were expressed as mean values accompanied by standard error (SEM). Statistical evaluation was performed using analysis of variance (ANOVA) in the SPSS software, with significance defined as $p < 0.05$.

Results and Discussion

The examination of the ethanol extract obtained from *S. alba* leaves revealed the existence of a diverse array of secondary metabolites. These include quinones, flavonoids, steroids, saponins, glycosides, phenolics, terpenoids, alkaloids, and tannins (refer to Table 1). The presence of these phytochemical constituents is integral in recognizing and comprehensively elucidating the extensive therapeutic potential inherent within the extract. It is plausible that these chemical compounds contribute, at least in part, to the observed antimalarial and antiplasmodial activities.

Antimalarial activity

The evaluation of the antimalarial efficacy of ethanol extracts sourced from *S. alba* leaves was conducted through a 4-day suppressive test regimen. Each experimental group received daily oral administrations of the plant extracts at varying concentrations (75, 150, and 300 mg/kg BW). Notably, the outcomes illustrated a marked suppression of *Plasmodium* parasites induced by the *S. alba* leaf extracts compared to the negative control group, particularly at higher dosage levels.

Table 1: Phytochemical analysis of the ethanolic extract derived from *S. alba* leaves

Secondary Metabolites	Results
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenolics	+
Steroids	-
Tannins	+
Saponins	+
Quinones	+
Glycosides	+

Notes: "+" = presence; "–" = absence.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Furthermore, a discernible dose-dependent reduction in parasite counts was observed following treatment with the ethanol extract of *S. alba* leaves. Nevertheless, complete parasite eradication was not attained in any of the groups administered with the *S. alba* leaf extracts. Conversely, the positive control group, treated with 36.4 mg/kg BW of artesunate, exhibited parasite suppression exceeding 95%. A comprehensive overview of the percentages of parasitemia and suppression observed is depicted in Figure 2.

The effective dose (ED_{50}) of the ethanol extract of *S. alba* leaves, necessary to inhibit 50% of parasite growth, was determined using the AAT Bioquest application. The results of the analysis and calculation of ED_{50} are presented in Figure 1.

Based on the graphical findings, the calculated ED_{50} value for the ethanol extract derived from *S. alba* leaves is determined to be 95.28 mg/kg BW. The classification of *in vivo* antiplasmodial activity is commonly delineated into three distinct categories: moderate (250–300 mg/kg BW), good (100–250 mg/kg BW), and very good (< 100 mg/kg BW).[2] In light of the ED_{50} value obtained, the ethanol extract sourced from *S. alba* leaves unequivocally falls within the classification of very good for *in vivo* antiplasmodial activity.

The efficacy of the ethanol extract of *S. alba* leaves against *Plasmodium* parasites is ascribed to the rich presence of diverse secondary metabolites encompassing terpenoids, phenolics, saponins, glycosides, flavonoids, tannins, alkaloids, and quinones within the extract. Among these secondary metabolites, quinones stand out for their noteworthy capacity to impede parasite growth, exemplifying their significant contribution to the antiplasmodial activity observed.[48]

Figure 2a vividly portrays the findings regarding the antiplasmodial activity of the ethanol extract sourced from *S. alba* leaves. After a duration of 4 days of treatment, a discernible trend emerges wherein the parasitemia percentage is notably diminished in the group receiving 300 mg/kg BW of the extract, signifying a clear dose-dependent correlation. Nonetheless, the parasitemia percentage in the positive control group registers significantly lower compared to the group subjected to the highest dosage of the extract ($p \leq 0.05$), thereby accentuating the distinction between the two treatment groups.

In Figure 2b, discernible trends emerge with higher doses of ethanol extracts sourced from *S. alba* leaves administered to test animals (mice), manifesting in a notable reduction in the percentage of parasitemia relative to the negative control group (*Plasmodium berghei*). Notably, the lowest percentage of parasitemia on day 4 was recorded at a dosage of 300 mg/kg BW, yielding an average parasitemia percentage of 4.61%. Furthermore, at a dosage of 150 mg/kg BW, the average percentage of parasitemia on day 4 stood at 6.41%, while at 75 mg/kg BW, it reached 8.76%. Moreover, the percentage of parasitemia for artesunate at a dosage of 36.4 mg/kg BW (positive control) on day 4 exhibited an average parasitemia percentage of 0.15%. These findings on the percentage of parasitemia facilitate the computation of the percentage of parasite growth inhibited by ethanol extracts of *S. alba* leaves and artesunate medications. Notably, the results of the percentage of parasite growth induced by ethanol extracts of *S. alba* leaves and artesunate at a dosage of 36.4 mg/kg BW (positive control) are visually depicted in Figure 2b.

The analysis from Figure 2b reveals a clear association between higher doses of ethanol extracts derived from *S. alba* leaves administered to test animals and diminished percentages of parasite growth within the host organisms. Notably, the administration of ethanol extracts of *S. alba* leaves at a dose of 300 mg/kg BW yielded the lowest percentage of parasite growth, with an average of 0.72%. Conversely, at doses of 150 mg/kg BW and 75 mg/kg BW, the average percentages of parasite growth were recorded at 1.19% and 1.78%, respectively. Noteworthy, these values surpassed the percentage of parasite growth observed with artesunate at a dose of 36.4 mg/kg BW (positive control), which exhibited an average parasite growth percentage of 0%. Additionally, after a 4-day treatment period, it becomes apparent that the percentage of parasite growth was significantly reduced in the group administered with 300 mg/kg BW of the extract compared to other dosage groups, thus indicating a dose-dependent correlation. However, the percentage of parasite growth was notably lower in the positive control group compared to the group treated with the highest extract dosage ($p \leq 0.05$).

The growth percentage findings serve as a foundation for computing the percentage of parasite growth inhibition induced by the ethanol extracts of *S. alba* leaves. Figure 2c elucidates the outcomes regarding the percentage of parasite growth inhibition by the ethanol extracts of *S. alba* leaves alongside artesunate at a dosage of 36.4 mg/kg BW (positive control), offering comprehensive insights into the comparative efficacy of these interventions.

Figure 2c elucidates a compelling relationship between the dosage of the ethanol extract of *S. alba* leaves administered to test animals and the subsequent percentages of inhibition of parasite growth observed within their bodies. Notably, the highest percentage of parasite growth inhibition induced by the ethanol extract of *S. alba* leaves was achieved at a dose of 300 mg/kg BW, manifesting an average percentage of parasite growth inhibition reaching 77.34%. Meanwhile, at dosages of 150 mg/kg BW and 75 mg/kg BW, the average percentages of parasite growth inhibition stood at 62.35% and 43.53%, respectively. However, despite these notable inhibitory effects, they were outstripped by the potent inhibitory prowess of artesunate at a dosage of 36.4 mg/kg BW (positive control), exhibiting an average percentage of parasite growth inhibition of 100%. Upon a 4-day treatment regimen, it becomes evident that the percentage of parasite growth inhibition was markedly higher in the group administered with 300 mg/kg BW of the extract compared to other dosage groups, thereby underscoring a discernible dose-dependent correlation. However, the percentage of parasite growth inhibition was notably higher in the positive control group compared to the group treated with the highest extract dosage ($p \leq 0.05$), indicating a substantial disparity in efficacy.

Parasites infecting red blood cells induce several changes, including enlarged red blood cell size, paleness of red blood cell color (decolorization), and the appearance of spots on certain stains.[30] There were observable differences in parasite morphology between all test groups and the normal controls over a 4-day observation period. The morphological variations observed across the test groups receiving the ethanol extract of *S. alba* leaves, in comparison to the normal controls (non-infected red blood cells with *Plasmodium berghei*), are distinguishable through distinctive alterations in parasite shape and the coloration of infected red blood cells. This comparison is visually depicted in the detailed illustrations presented in Figure 3 below.

The graph provided in Figure 2c portrays the percentage of parasite inhibition, delineating a notable disparity in effectiveness across different dosage regimens of the ethanol extract of *S. alba* leaves. Specifically, it highlights that the administration of the ethanol extract at a dosage of 300 mg/kg BW elicited the most substantial percentage of inhibition in contrast to dosages of 75 mg/kg BW and 150 mg/kg BW. On the fourth day of extract administration, it demonstrated an inhibition percentage of 77.34%. Additionally, the graph in Figure 2b above shows that the ethanol extract of *S. alba* leaves at all therapeutic doses, along with artesunate at a dose of 36.4 mg/kg BW (positive control), exhibited antiplasmodial activity against *Plasmodium berghei*. This activity involves inhibiting the growth of parasites on red blood cells. The assessment of the effective dose required to impede 50% of parasite development (ED_{50}) induced by the ethanol extract of *S. alba* leaves was conducted using the AAT Bioquest software.

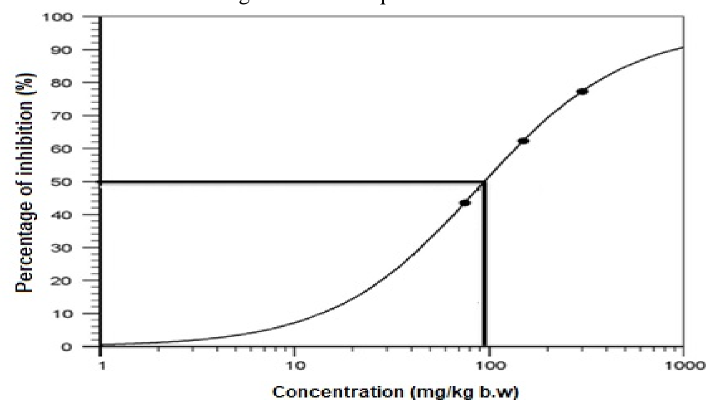
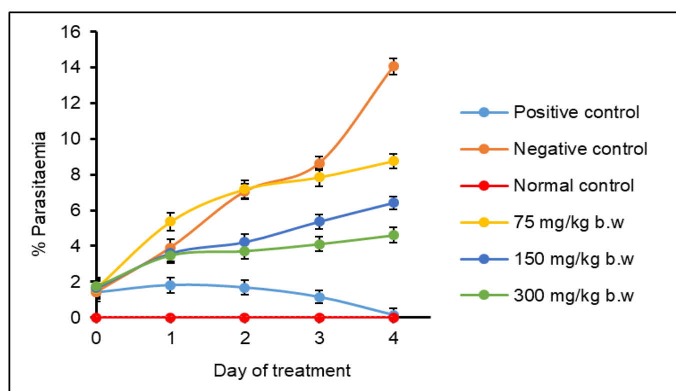


Figure 1: The growth inhibition of *P. berghei* with the addition of ethanolic extract of *S. alba* leaves

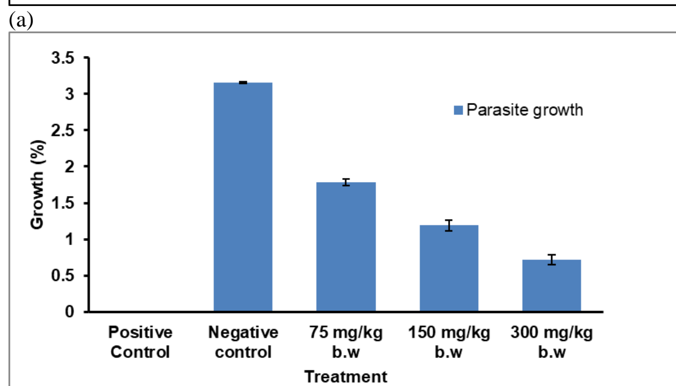
© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

The findings, along with the calculated ED_{50}, are comprehensively depicted in Figure 1, providing invaluable insights into the dosage threshold crucial for inhibiting parasite proliferation. The investigation of antiplasmodial activity can involve assessing the suppression of the *Plasmodium* life cycle. Malaria's pathophysiology is primarily linked to the blood stage, where sporozoites transmitted through mosquito bites infect hepatocytes and proliferate through the circulatory system.[2,6,48] As a result of its morphology, the early trophozoite stage is commonly referred to as the "ring form." Following a 24-hour interval, blood specimens were procured and subjected to Giemsa staining to scrutinize the impact of the extract on the life cycle of parasitized *Plasmodium*. The administration of the *S. alba* leaf extract showcased a notable inhibition of the transition from the ring stage to the schizont stage, as visually represented in Figure 3, shedding light on its efficacy in impeding crucial stages of the parasite's life cycle. At a concentration of 300 µg/mL of extract, no schizont stage production was observed, indicating disruption of the *Plasmodium*'s life cycle as the schizonts were eliminated.



(a)



(b)

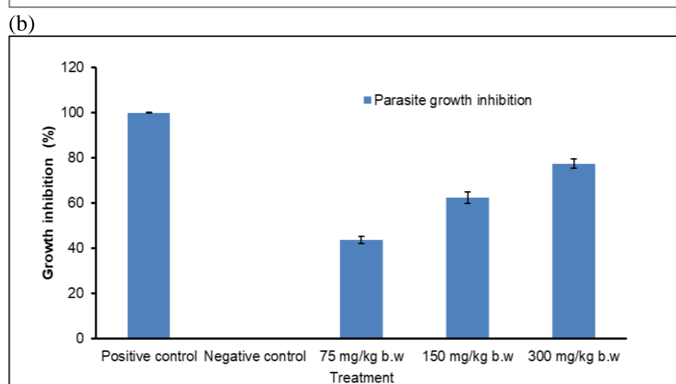


Figure 2: (a) The percentage of parasitemia determined by an erythrocytic-antiplasmodial assay of *S. alba* leaf ethanolic extracts; (b) The impact of ethanolic extracts derived from *S. alba* leaves on the percentage of parasite growth following a 4-day treatment period; (c) the impact of ethanolic extracts obtained from *S. alba* leaves on the percentage inhibition of parasite growth after 4 days of treatment.

However, even at lower concentrations, trophozoites and schizonts persisted with morphological defects. Treatment of blood samples with 300 µg/mL of the extract revealed that while some schizonts survived with defects, several were destroyed. The *Plasmodium*'s life cycle remained unaffected by lower concentrations of extract treatment on blood samples.

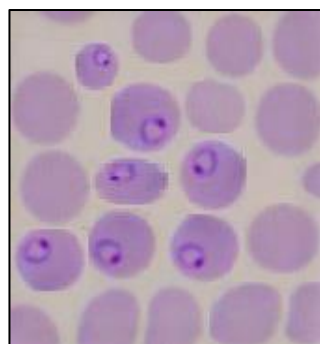
The phytochemical screening unveiled a diverse array of compounds within the ethanol extract acquired from *S. alba* leaves, encompassing saponins, glycosides, quinones, alkaloids, terpenoids, polyphenols, flavonoids, and tannins. These constituents, well-documented in plants, such as flavonoids, quinones, alkaloids, and triterpenoids, have been associated with antiplasmodial efficacy.[2,6,35–39,43] This study postulates that the presence of these chemical entities within the extract and its fractions could contribute, at least in part, to the observed antimalarial and antiplasmodial properties. Furthermore, the methanol and ethanol extracts of *S. alba* leaves may exhibit a regulatory effect on protozoans, potentially acting as biological response modifiers akin to antimalarial agents.[6] Consequently, *S. alba* emerges as a promising reservoir for natural antimalarial therapeutics, echoing its longstanding utilization in traditional medicine. The antimalarial potential of *S. alba* leaves may derive from the accumulation of phytochemical constituents capable of inducing red blood cell oxidation elevation or inhibiting protein synthesis, underpinning its therapeutic efficacy.[2,26,43]

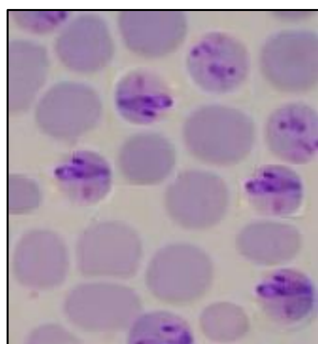
In the progression of drug development sourced from natural origins, meticulous scrutiny involving both *in vitro* and *in vivo* assessments remains indispensable for gauging the potency and safety profile of a novel pharmaceutical agent.[2,32,33,43] Previous investigations from our research team, focusing on *in vitro* evaluations, unveiled the compelling antimalarial efficacy of the ethanol extract originating from *S. alba* leaves against the *P. berghei* strain, a formidable adversary due to its resistance to chloroquine, with an IC_{50} value recorded at 30 µg/ml. This promising outcome instigated a comprehensive exploration into its *in vivo* antimalarial attributes, as elucidated in the present study. The establishment of an *in vivo* model serves as a pivotal platform for discerning potential prodrug effects, deciphering the intricate role of the immune system in combating infections, and ensuring the pharmacological safety profile of the drug candidate before progressing to clinical trials.[2,4,6,33,43] In this investigation, our research employed ICR mice harboring wild-type *P. berghei* ANKA infections as the model organism to delve into antimalarial properties. The utilization of the *P. berghei* ANKA infection model stands as a well-established approach to induce malaria within a mouse setting, facilitating the execution of a 4-day suppressive test. This particular strain's propensity to sequester within the microvasculature renders it invaluable for mimicking severe malaria manifestations, a characteristic pivotal for comprehensive drug assessment.[2,32] Over the years, this strain has served as a cornerstone for evaluating a myriad of antimalarial agents, including halofantrine, mefloquine, chloroquine, and artemisinin derivatives.[2,6,32,33,43] The adoption of the 4-day suppressive test methodology was dictated by its widespread use in appraising the antimalarial efficacy of novel therapeutics during the nascent stages of infection. Central to this evaluation is the computation of the suppression percentage of blood parasitemia, an indispensable metric guiding the assessment protocol.[3,32]

Prior investigations have underscored the multifaceted therapeutic potential of the ethanol extract and chemical constituents derived from *S. alba* leaves, exhibiting pronounced antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective attributes.[6,43,52] In an animal model setting, the dose-dependent antimalarial efficacy of the ethanol extract of *S. alba* leaves was corroborated, manifesting a notable 77.34% reduction in *P. berghei* parasitemia upon administration at a high dosage (300 mg/kg BW). The detection of terpenoids, steroids, saponins, flavonoids, alkaloids, glycosides, phenolics, tannins, and quinones within the ethanol extract substantiates congruent findings from extant literature.[6,43,48] Consequently, quinones and flavonoids have emerged as pivotal contributors to the antimalarial potency of the ethanol extract of *S. alba* leaves. Recent scholarly discourse posits that these bioactive compounds exert their antimalarial effects by impeding the invasion of *P. berghei* into infected erythrocytes.[2,6,43] Notably, quinones and flavonoids were identified as the predominant constituents within the ethanol extract of *S. alba* leaves.[6]

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)


Ring stage


Trophozoite stage

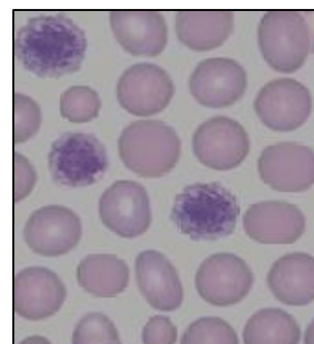

Schizont stage

Figure 3: The effect of ethanol extract of *S. alba* leaves (300 mg/kg BW) on the life cycle of *Plasmodium* (1000× Magnification).
Notes: *S. alba* leaves ethanol extract has activity in hindering the development of ring stage to the schizonts stage. The schizonts stage formation was not found at 300 mg/kg BW of extract which can be assumed that the life cycle of plasmodium has been affected since the schizonts has been destroyed.

This condition arises from the intricate interplay of diverse phytochemical constituents, which potentially harbor antimalarial properties. These constituents are believed to play a pivotal role in driving the observed antiplasmodial activity. Distinctively, certain plant-derived compounds, including quinones, have garnered attention for their antioxidant capacities, which could augment schizonticidal activity by modulating intricate cellular signaling pathways.[6] This phenomenon finds support in the literature, with compounds like kempferol-3-O-rhamnoside, quercetin, methyl gallate, and gallic acid being implicated in such activities.[2,6,43,50] Elevated levels of free radicals, commonly associated with malaria pathogenesis, have been linked to severe malaria complications. Therefore, it is conceivable that the action of the extract involves the presence of antioxidant-active phenolics and quinones.[2,6] Quinones, in particular, have been shown to possess antiplasmodial activity through mechanisms such as chelation with the parasite's nucleic acid base pairing.[2,6] The presence of quinone molecules in the plant extract could plausibly contribute to the plasmodial activity observed in the ethanol extract of *S. alba* leaves, shedding light on the underlying mechanism of the extract's antiplasmodial effects.

Phytochemicals constitute vital constituents of medicinal plants and contribute to a diverse array of bioactivities. Antiplasmodial activity has been identified in numerous plants harboring a spectrum of phytochemicals as their bioactive agents.[11–18] Although the mechanism of action of the leaf extract was not investigated in this study, several elements found in this study have been associated with antiplasmodial activity through various pathways. Alkaloids, flavonoids, and terpenoids prevalent in *S. alba* have been implicated in antiplasmodial action.[2,6,40–45]

Trophozoite growth involves metabolic processes, including the utilization of host cytoplasm. The exoerythrocytic schizogony, or schizont stage,[2,6,43] marks the culmination of the trophic phase, characterized by multiple rounds of nuclear division without cytokinesis.[2] Parasite growth is predominant during the ring stage at 0 hours. Beyond 24 hours, parasites progress into trophozoite and schizont stages; however, morphological abnormalities become evident, rendering the parasites appear as deceased cells upon microscopic examination (refer to Figure 3). A static ring stage was observed at extract concentrations exceeding 300 µg/ml, indicative of growth inhibition or detrimental effects. According to the findings, ethanol extracts of *S. alba* leaves exert activity on the *Plasmodium*'s life cycle.

The ethanol extracts derived from *S. alba* leaves demonstrate significant antiplasmodial activity, as indicated by notable reductions in parasitemia levels, percentage of inhibition, and inhibition of *Plasmodium* growth. The present study highlights the robust antimalarial potential exhibited by ethanol extracts derived from *S. alba* leaves.[6,43] These findings align with previous research on this genus, which has unveiled a spectrum of biological actions exhibited by its crude extracts and compounds, including anticancer, antioxidant, antimicrobial, and anti-inflammatory properties.[6,48] Moreover, accumulating evidence suggests that compounds extracted from *S. alba* possess antiplasmodial activity, thereby positioning it as a valuable and potent source of natural antimalarial agents.

Conclusion

This study revealed that ethanol extracts derived from *S. alba* leaves exhibited significant antiplasmodial efficacy, as evidenced by their ability to decrease parasitemia levels, induce a notable percentage of inhibition, and inhibit *Plasmodium* growth. Thus, *S. alba* leaves emerged as a promising natural reservoir of antimalarial agents in this study. Furthermore, the ethanol extract derived from *S. alba* leaves exhibited a diverse array of phytochemical constituents and underwent rigorous scientific validation to confirm its antiplasmodial efficacy.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors extend their sincere appreciation to Padjadjaran University for its generous support of this project through the Academic Leadership Grant awarded for the year 2022. Grant Number 2203/UN6.3.1/PT.00/2022.

References

1. Phuwajaroanpong A, Chaniad P, Horata N, Muangchanburee S, Kaewdana K, Punsawad C. *In vitro* and *in vivo* antimalarial activities and toxicological assessment of *Pogostemon cablin* (Blanco) Benth. J. Evidence-Based Integrative Med. 2020;25:1-11.
2. Chaniad P, Techarang T, Phuwajaroanpong A, Na-ek P,Viriyavejakul P, Punsawad C. *In vivo* antimalarial activity and toxicity study of extracts of *Tagetes erecta* L. and *Synedrella nodiflora* (L.) Gaertn. from the Asteraceae Family. Evidence-Based Comple & Alterna Med. 2021; 1; 1-9.
3. Peters W, Portus JH, Robinson BL. The chemotherapy of rodent malaria, XXII. Annals of Trop Med & Parasit. 1975; 69(2):155–171.
4. Chaerunisaaa AY, Susilawati Y, Muhaimin M, Milanda T, Hendriani R, Subarnas A. Antibacterial activity and subchronic toxicity of *Cassia fistula* L. barks in rats. Toxicol Rep. 2020; 7:649-657.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

5. Retnosari D, Purnobasuki H, Supriyanto A. Antimalarial Activity of Crude Bark Extract of *Pterocarpus indicus* Willd. against *Plasmodium falciparum* Strain 3D7. Trop J Nat Prod Res. 2023; 7(9):3893-3897. http://www.doi.org/10.26538/tjnpr/v7i9.6.
6. Muhaimin, Yusnaidar, Syahri W, Latief M, Utami A, Bemis R, Amanda H, Heriyanti, Chaerunisaa AY. Screening and potential analysis of methanolic leaf extract of Mangrove plants at east coast Sumatera as repellent against *Aedes aegypti*. J Pharm Sci Res. 2018; 10(9): 2228-2231.
7. Fadipe LA, Ajemba C, Lawal BA, Ahmadu AA, Ibikunle GF. Phytochemical and *In-vivo* Antimalarial Investigations of *Dichrostachys cinerea* (L.) Wight & Arn. (Fabaceae) Root Bark. Trop J Nat Prod Res. 2020; 4(11):1007-1014. doi.org/10.26538/tjnpr/v4i11.28.
8. Habibi P, Shi Y, Fatima Grossi-de-Sa M, Khan I. Plants as Sources of Natural and Recombinant Antimalaria Agents. Mol Biotechnol. 2022; 64(11):1177-1197.
9. Berthi W, González A, Rios A, Blair S, Cogollo Á, Pabón A. Anti-plasmodial Fffect of Plant Extracts from *Picrolemma huberi* and *Picramnia latifolia*. Malar J. 2018; 17(1):151.
10. Uzor PF. Alkaloids from Plants with Antimalarial Activity: A Review of Recent Studies. Evid Based Complement Alterna Med. 2020; 20(1):1-17.
11. Syafrizal, Ramadhan R, Kusuma IW, Egra S, Shimizu K, Kanzaki M, Arung ET. Diversity and honey properties of stingless bees from meliponiculture in East and North Kalimantan, Indonesia. Biodiv J Biologic Div, 2020; 21(10):4623-4630.
12. Shija KM, Nondo RSO, Mloka D, Sangeda RZ, Bwire GM. Effects of Lemon Decoction on Malaria Parasite Clearance and Selected Hematological Parameters in *Plasmodium berghei* ANKA Infected Mice. BMC Complement Med Ther. 2020; 20(1):24.
13. Ihekwereme CP, Melidem CO, Maduka IC, Okoyeh JN. *In Vivo* Antiplasmodial and Toxicological Effects of Extracts of Fruit Pulp of *Chrysophyllum albidium* G. Don (Sapotaceae). Trop J Nat Prod Res. 2018; 2(3):126-131. doi.org/10.26538/tjnpr/v2i3.5.
14. Ojo SKS, Udewena LU, Durodola OT, Olarinoye OO, Ariyo OO, Herbert SJ, Lawal AM. Antibacterial Activity of *Bryophyllum pinnatum* and *Rauvolfia vomitoria* on Neonatal Group B Streptococcus. Trop J Nat Prod Res. 2024; 8(2):6457-6464. http://www.doi.org/10.26538/tjnpr/v8i2.39.
15. Laksemi DA, Sukrama ID, Suwanti LT, Sudarmaja M, Damayanti PA, Tunas IK, Wiryanthini IA, Linawati NM. A Comprehensive Review on Medicinal Plants Potentially as Antimalarial. Trop J Nat Prod Res, 2022; 6(3), 287–298.
16. Al-Aamri MS, Al-Abousi NM, Al-Jabri SS, Alam T, Khan SA. Chemical Composition and *in Vitro* Antioxidant and Antimicrobial Activity of the Essential Oil of *Citrus aurantifolia* L. Leaves grown in Eastern Oman. J Taibah Univ Med Sci. 2018; 13(2):108-112.
17. Uchenna BA, Abdullahi M, Yusuf KA, Olofu OE. Antimalarial activity of crude extract and fractions of *Phyllanthus amarus* in *Plasmodium berghei* infected mice. Eur J Med Plants. 2018; 24: 1-11.
18. Muhaimin, Syamsurizal, Chaerunisaa AY, Sinaga MS. Eusiderin I from *Eusideroxylon zwagery* as antifungal agent against plant pathogenic fungus. Int J ChemTech Res. 2016; 9(5): 418-424.
19. Chaerunisaa AY, Muhaimin. Comparative study on release of two drugs in fixed dose combination using zeroorder and first derivative spectrophotometry. Int J PharmTech Res. 2016; 9(12): 581-590.
20. Mulenga MC, Sitali L, Ciubotariu II, Hawela MB, Hamainza B, Chipeta J, Mharakurwa S. Decreased prevalence of the *Plasmodium falciparum* Pfcrt K76T and Pfmdr1 and N86Y mutations post-chloroquine treatment withdrawal in Katete District, Eastern Zambia. Malaria J. 2021; 20: 329.
21. Taek MM, Tukan GD, Prajogo BEW, Agil M. Antiplasmodial activity and phytochemical constituents of selected antimalarial plants used by native people in West Timor Indonesia. Turkish J Pharm Sci. 2021; 18(1):80-90.
22. Nwonuma CO, Balogun EA, Gyebi GA. Evaluation of antimalarial activity of ethanolic extract of *Annona muricata* L.: an *in vivo* and an *in silico* Approach. J Evid Based Integr Med. 2023;28: 1-10.
23. Abouelela ME, Abdelhamid RA, Orabi MAA. Phytochemical constituents of plant species of Pterocarpus (F: Leguminosae): a review. Int J Pharmacogn Phytochem Res. 2019; 11(4): 264-281.
24. WHO. Malaria world report. World Health Organization, Geneva, Switzerland: WHO Press; 2019; 1-50.
25. Czechowski T, Rinaldi MA, Famodimu MT, Veelen MV. Larson TR. Thilo W. Rathbone DA. Harvey D. Horrocks P. Graham IA. Flavonoid versus Artemisinin Anti-malarial Activity in *Artemisia annua* Whole-Leaf Extracts. Front Plant Sci. 2019; 10(984):1-11.
26. Hailesilase GG, Rajeshwar Y, Hailu GS, Sibhat GG, Bitew H. *In vivo* antimalarial evaluation of crude extract, solvent fractions, and TLC-isolated compounds from *Olea europaea* Linn subsp. cuspidata (Oleaceae). Evid Based Complement Alter Med. 2020: 15-20.
27. Saivaraj S. Antimicrobial activity of natural dyes obtained from *Pterocarpus indicus* Wild. barks. Asian J Pharm Res Dev. 2018; 6(2): 6-8.
28. Abdussalam US, Allyu M, Maje IM. *In vivo* antiplasmodial activity of ethanol leaf extract of *Marrubium vulgare* L. (Lamiaceae) in *Plasmodium berghei*-berghei infected mice. Trop J Nat Prod Res. 2018; 2(3): 132-135.
29. Altharawi A, Riadi Y, Tahir Ul Qamar M. An in silico quest for next-generation antimalarial drugs by targeting *Plasmodium falciparum* hexose transporter protein: a multipronged approach. J Biomol Struct Dyn. 2023;1–10.
30. Ogbeide OK, Dickson VO, Jebba RD, Owhiroro DA, Olaoluwa MO, Imieje VO, Erharuyi O, Owolabi BJ, Fasinu PS, Falodun A. Antiplasmodial and acute toxicity studies of fractions and cassane-type diterpenoids from the stem bark of *Caesalpinia pulcherrima* (L.) Sw. Trop J Nat Prod Res. 2018; 2(4): 179-184.
31. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* antiinflammatory activities of *Severinia buxifolia*. J Food Qua. 2019; 19(1): 1-10.
32. Pippione AC, Sainas S, Goyal P, Fritzson I, Cassiano GC, Giraudo A, Giorgis M, Tavella TA, Bagnati R, Rolando B, Caing-Carlsson R, Costa FTM, Andrade CH, Al-Karadaghi S, Boschi D, Friemann R, Lolli ML. Hydroxyazole scaffoldbased *Plasmodium falciparum* dihydroorotate dehydrogenase inhibitors: Synthesis, biological evaluation and X-ray structural studies. Eur J Med Chem. 2019;163:266–80.
33. Owoloye AJ, Ligali FC, Enejoh OA, Musa AZ, Aina O, Idowu ET, Oyebola KM. Molecular docking, simulation and binding free energy analysis of small molecules as PfHT1 inhibitors. PloS One. 2022;17(8):e0268269.
34. Czechowski T, Rinaldi MA, Famodimu MT, Veelen MV. Larson TR. Thilo W. Rathbone DA. Harvey D. Horrocks P. Graham IA. Flavonoid versus Artemisinin Anti-malarial Activity in *Artemisia annua* Whole-Leaf Extracts. Front Plant Sci. 2019; 10(984):1-11.
35. Tajuddeen N, Van Heerden FR. Antiplasmodial natural products: an update. Malaria J 2019; 18(1): 404.
36. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and *in vitro* antiinflammatory activities of *Severinia buxifolia*. J Food Qua. 2019; 1-15.

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

37. Siddiqui FA, Boonhok R, Cabrera M, Mbenda HGN, Wang M, Min H, Liang X, Qin J, Zhu X, Miao J, Cao Y, Cui L. Role of *Plasmodium falciparum* Kelch 13 Protein Mutations in *P. falciparum* Populations from Northeastern Myanmar in Mediating Artemisinin Resistance. mBio. 2020;11(1):11-19.
38. Heller LE, Roepe PD. Quantification of Free Ferriprotoporphyrin IX Heme and Hemozoin for Artemisinin Sensitive versus Delayed Clearance Phenotype *Plasmodium falciparum* Malarial Parasites. Biochemistry. 2018;57(51):6927–6934.
39. Heller LE, Goggins E, Roepe PD. Dihydroartemisinin Ferriprotoporphyrin IX Adduct Abundance in *Plasmodium falciparum* Malarial Parasites and the Relationship to Emerging Artemisinin Resistance. Biochemistry. 2018;57(51):6935–6945.
40. Ross LS, Lafuente-Monasterio MJ, Sakata-Kato T, Mandt REK, Gamo FJ, Wirth DF, Lukens AK. Identification of Collateral Sensitivity to Dihydroorotate Dehydrogenase Inhibitors in *Plasmodium falciparum*. ACS Infect Dis. 2018;4(4):508–515.
41. Chagas CM, Moss S, Alisaraie L. Drug metabolites and their effects on the development of adverse reactions: Revisiting Lipinski's Rule of Five. Int J Pharm. 2018;549(1–2):133–49.
42. Boschi D, Pippione AC, Sainas S, Lolli ML. Dihydroorotate dehydrogenase inhibitors in anti-infective drug research. Eur J Med Chem. 2019;183:111-118.
43. Muhaimin M, Yusnaidar Y, Syahri W, Latief M, Putri RD, Utami A, et al. Antiplasmodial Activity of Ethanolic Extract of *Macaranga Gigantea* Leaf and Its Major Constituent. Pharmacog J. 2019;11(6):1181-1188.
44. Chaniad P, Techarang T, Phuwajaroanpong A, Na-ek P, Viriyavejakul P, Punsawad C. *In Vivo* Antimalarial Activity and Toxicity Study of Extracts of *Tagetes erecta* L. and *Synedrella nodiflora* (L.) Gaertn. from the Asteraceae Family. Evidence-Based Complemen & Alternat Med. 2021; 902-912.
45. Nsanzabana C. Resistance to artemisinin combination therapies (ACTs): do not forget the partner drug. Trop Med & Infect Dis. 2019; 4(1):26.
46. Juhairiyah J, Andiarsa D, Indriyati L, Ridha MR, Prasodjo RS, Dhewantara PW. Spatial analysis of malaria in Kotabaru, South Kalimantan, Indonesia: an evaluation to guide elimination strategies. Trans R Soc Trop Med Hyg. 2021;115(5):500–511.
47. Carmichael N, Day PJR. Cell Surface Transporters and Novel Drug Developments. Front Pharmacol. 2022;13: 938-946.
48. Muhaimin M, Yusnaidar Y, Syahri W, Latief M, Chaerunisaa AY. Microencapsulation of *Macaranga gigantea* Leaf Extracts: Production and Characterization. Pharmacog. J. 2020; 12 (4):716-724.
49. Astuti P, Rollando R, Wahyuono S, Nurrochmad A. Antimicrobial activities of isoprene compounds produced by an endophytic fungus isolated from the leaves of *Coleus amboinicus* Lour. J Pharm Pharmacogn Res. 2020;8(4):280–9.
50. Okokon JE, Antia BS, Mohanakrishnan D, Sahal D. Antimalarial and antiplasmodial activity of husk extract and fractions of *Zea mays*. Pharm Biol. 2017; 55(1): 1394–1400.
51. Hariono M, Julianus J, Djunarko I, Hidayat I, Adelya L, Indayani F, Auw Z. Namba G, Hariyono P. The Future of *Carica papaya* Leaf Extract as an Herbal Medicine Product. Mol Basel Switz. 2021;26(22):6922.
52. Muhaimin, Yusnaidar, Syahri W, Latief M, Utami A, Bemis R, et al. Screening and potential analysis of methanolic leaf extract of Mangrove plants at east coast Sumatera as repellent against *Aedes aegypti*. J Pharm Sci Res. 2018;10(9):2228-31

© 2023 the authors. This work is licensed under the Creative Commons Attribution 4.0 International License