Abstract— Malaria is a global public health concern due to the increase of resistance to antimalarials, therefore the search for new antimalarials is of importance. This study was conducted to explore the antimalarial activity of Swietenia mahagoni and Kibatalia arborea in the form of crude methanolic extract. The plants were collected from Klaten-Central Java, and Batu-East Java, Indonesia. In vitro antimalarial assay was done against Plasmodium falciparum. Phytochemical studies were done chemically using Dragendorff’s reagent and spectroscopically using the ¹H-NMR technique. Results show that the extract indicated positive antimalarial activity. Preliminary chemotype studies revealed the extract constitutes alkaloid compounds in which proton NMR indicated a typical aromatic alkaloid molecular structure. In conclusion, S. mahagoni K. arborea are potential for an antimalarial agent and further studies are necessary to obtain the bioactive compounds responsible for the claimed activity.

Keywords— Indonesian medicinal plant; Antimalaria; Swietenia mahagoni; Kibatalia arborea

I. INTRODUCTION

Malaria is an infection caused by Plasmodium parasites that are transmitted by mosquitos. Therefore, it spreads across tropical and sub-tropical regions of the world, including Indonesia. After having been scrutinized since 2008, just only a few years ago, Plasmodium knowlesi was annexed as the fifth parasite to cause malaria in humans [1–3]. It has been infectious to the human population since approximately between 10,000 and 100,000 years ago in Africa [4]. This infectious disease has remained an important public health problem. According to the latest World Health Organization’s (WHO) report on malaria, about 219 million people worldwide in 2017 were infected with this disease (95% confidence interval [CI] 203-262 million). The global deaths caused by malaria in 2017 were estimated to be 435,000, with 61% (266,000) were accounted for the most vulnerable group, the children aged under 5 [5]. Although between 2000 and 2015 the world had seen the reduction of cases and deaths due to malaria, the progress has been halted since then [6]. This could be a hindrance to reach the 2030 goal which is a 90% reduction in malaria incidence and mortality [7].

Several factors are identified to be the problems for malaria prevention and control including lower efficacy of the vaccine, resistance to the insecticide used for a bed net, and resistance to available antimalarials [7, 9]. The report on the results of a phase III clinical trial of a malaria vaccine candidate (the RTS, S/AS01) in seven countries of Sub-Saharan Africa was disappointing because the vaccine only gave a small protection for children aged 5-17 months and even did not show protection for the younger infants [10-11]. The vaccine itself had been developed for about 25 years before it went to the phase III trial [10]. Although a recent Cochrane systematic review suggests that the evidence of benefits from using insecticide-treated bed nets on the reduction of all-cause child mortality and prevalence of P falciparum malaria remains strong, this development poses a serious threat to global malaria control [12]. In addition to problems of malaria prevention and control, there has been the resistance of P falciparum to antimalarials, either for monotherapy or in combination. Historically, the resistance of P falciparum and P vivax are closely correlated with Southeast Asia. Menard and Dondorp [13] summarized that partial artesinin resistance in P falciparum has firstly occurred in Cambodia followed by other countries of the Greater Mekong Subregion. The resistance ofartesinin and its partner drugs in the Artemisinin Combination Therapy (ACT) were firstly identified in Cambodia and the Thai-Myanmar border. The resistance in P vivax to chloroquine was reported to be emerging in Indonesia. All aforementioned problems therefore demand the need of finding new antimalarials. Malarial endemicity in Indonesia still remains a serious health burden (Fig. 1).

Fig. 1. Endemicity class predictions of Plasmodium falciparum malaria PfPR2–10 in Indonesia [8].
In history, the discoveries of antimalarials are closely related to the use of natural products. Quinine was first isolated from the chinchona tree in 1820 by a European scientist and artemisinin was first isolated from Artemisia annua in 1971 by a Chinese scientist [14-15]. The discovery of their activity as potent antimalarials created a big hope for malaria control. The extensive laboratory research on Indonesian medicinal plants has been started in the 1970s in which berberine alkaloid was isolated from Arcangelisia Flava Merr. From more than 7,000 records on Indonesian medicinal plants, less than 200 species have been studied focused on anti-infective drug discovery [16]. This including, exploration pursuing antimalarial agents from Indonesian medicinal plants used by the people in endemic malaria [17]. A good example for a source of antiparasitic agents is seeds of Swietenia mahagoni and leaves of Kibatalia arborea.

Different from Swietenia macrophylla, the S. mahagoni grows with smaller leaves sizes. The bitter taste seeds have been prepared traditionally as antitmetabolic medicament by the indigenous people of Indonesia including antiabetic, antirheumatic and antihypertensive agents [18-19]. In addition, the seed has been also prepared to treat malaria and eczema [18].

Exudate producing Apocynaceae tree, Kibatalia arborea are common in Java island along with wood source Kopsia arborea. Ethnopharmacological data recorded the plant has been used by the indigenous people of Indonesia as antiparasitic agents, especially as anthelmintic agents [18].

In this study, these two medicinal plants were evaluated for their phytochemical, spectral and biological activities against pathogenic parasite, Plasmodium falciparum 3D7.

B. Extraction
Samples (1 g, each) were powdered followed by soaking with methanol (10 mL) and sonicated for 1 hour. The mixture was filtered and was then dried.

C. Phytochemical study
Thin Layer Chromatography (TLC) based phytochemical study was employed with visualizing reagent Vanillin-H₂SO₄ staining agent for general chemotype which red color indicated for phenols present, grey for sugar, purple for terpenoids. Dragendorff’s reagent was used to detect alkaloid which red-orange color give a positive result.

C. ¹H-NMR study
Each sample (5 mg) was dried through a high vacuum apparatus under the silica gel chamber before dissolving in CD₃OD. The ¹H-NMR was recorded in Jeol NMR 400 MHz.

D. Anti-plasmodium bioassay
Each sample (1 mg) was dissolved in DMSO (100 μL) followed by serial dilution to obtain 1000, 100, 10, 1 ug/mL. Plasmodium falciparum 3D7 strain parasite was used at ring stadium with parasitemia level of ± 1%. Sample (2 μL) with serial concentration were loaded into 96 well plate followed by parasite (198 μL) addition to form last concentration as 100, 10, 1, 0.1 and 0.01 μg/mL. The plate was treated with mixed gases (5% O₂, 5% CO₂ and 90% N₂) prior incubation at 37°C. The culture was collected, stripped and stained with 10% Giemsa reagent. Percentage inhibition was calculated based on formula 1.

\[
\text{% Inhibition}=100\% - \left(\frac{\text{Xu}}{\text{Xk}}\right)\times100\% \quad (1)
\]

Note Xu=% growth of sample, Xk=%growth of the negative control. IC₅₀ was obtained as the concentration which inhibits 50% growth of the parasite.

III. RESULTS and DISCUSSION

Strong acid-Vanilin reagent on TLC based phytochemical analysis on the Swietenia mahagoni seeds produced intense red color with no significant purple color production. These indicated the presence of phenolic constituents. Alkaloid was detected as there was a strong orange color produced by Dragendorff’s reagent test.

HPLC chromatogram (Fig. 3, top) showed the crude methanol extract constituted of medium polarity components in which alkaloid fractionation clearly left at least four alkaloid presence (Fig. 3).

Through spectral comparison of the proton NMR spectrum of crude methanol extract (Fig. 4) against spectral data of previously reported limonoids from twigs and leaves of Chinese origin S. mahagoni, swiemaohinosins A and B [20], dereplication protocol clearly distinguished inexistence of these major limonoid components in the seed sample. On the other hand, 15 limonoids were previously reported from S. macrophylla [21].
No alkaloid was previously reported on *S. mahagoni* and only one alkaloid, 3,6,7-trimethoxy-4-methyl-1,2,3,4-tetrahydroisoquinoline was reported from Indonesian *S. macrophylla* seed with limited information on its molecular structure elucidation [22]. Hence, further dereplication is necessary for obtaining understudied or novel compounds.

![Fig. 3. HPLC profile of crude methanol extract of seed of *Swietenia mahagoni* seeds (top) and its alkaloid fraction (below). The profiles were obtained from 10-90% acetonitrile in water development recorded at 254 nm.](image)

![Fig. 4. 1H-NMR spectrum of crude methanol extract of leaves of *Swietenia mahagoni*.](image)

Fig. 3. HPLC profile of crude methanol extract of seed of *Swietenia mahagoni* seeds (top) and its alkaloid fraction (below). The profiles were obtained from 10-90% acetonitrile in water development recorded at 254 nm.

![Fig. 5. HPLC profile of crude methanol extract of leaves of *Kibatalia arborea* obtained from 10-90% acetonitrile in water development recorded at 254 nm.](image)

![Fig. 6. 1H-NMR spectrum of crude methanol extract of leaves of *Kibatalia arborea*.](image)

![Fig. 6. 1H-NMR spectrum of crude methanol extract of leaves of *Kibatalia arborea*.](image)

Fig. 4. 1H-NMR spectrum of crude methanol extract of leaves of *Swietenia mahagoni*.

Fig. 5. HPLC profile of crude methanol extract of leaves of *Kibatalia arborea* obtained from 10-90% acetonitrile in water development recorded at 254 nm.

![Fig. 5. HPLC profile of crude methanol extract of leaves of *Kibatalia arborea* obtained from 10-90% acetonitrile in water development recorded at 254 nm.](image)

The proton NMR spectrum (Fig. 6) illustrated mixture chemotype with rich aromatic, sugar moiety and sp$^3$ hybridized carbon chain. There is no previous report regarding the phytochemical study on this species. However, steroidal alkaloid was previously reported from other *Kibatalia* species. Aromatic rich compounds have not been reported form the *Kibatalia* genus [23].

![Fig. 6. 1H-NMR spectrum of crude methanol extract of leaves of *Kibatalia arborea*.](image)

Fig. 6. 1H-NMR spectrum of crude methanol extract of leaves of *Kibatalia arborea*.

This study reported the antimalarial activity of *Kibatalia* species, in which crude methanol extract of leaves of *Kibatalia arborea* indicated a significant anti-plasmodium activity against *Plasmodium falciparum* 3D7 with an IC$_{50}$ value of 12.37 µg/mL.

**IV. CONCLUSION**

The study revealed antimalarial potency of seeds of *Swietenia mahagoni* and leaves of *Kibatalia arborea* with significant anti-plasmodial activity. Simple dereplication eased to focus on understudied components of *Swietenia mahagoni* seeds and *Kibatalia arborea* leaves. Further study is necessary to gain pure single compounds which responsible for the antimalarial activity.

**ACKNOWLEDGMENT**

The research was funded by the DRPM research grant from KEMENRISTEKDIKTI of the Republic of Indonesia.
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