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Preliminary Study on Antimalarial Agent From Indonesian *Swietenia Mahagoni* and *Kibatalia Arborea*

Bawon Triatmoko[1], Dwi Koko Pratoko[1], Antonius Nugraha Widhi Pratama[1], Yoshinta Debby Purnomo[1], Tinton Agung Laksono[1] and Ari Satia Nugraha[1*]
[1]Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, University of Jember, Jember, 68121, Indonesia
[*]E-mail: arisatia@unej.ac.id

***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 ^{1}H-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 artemisinin resistance in *P falciparum* has firstly occurred in Cambodia followed by other countries of the Greater Mekong Subregion. The resistance of artemisinin 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).

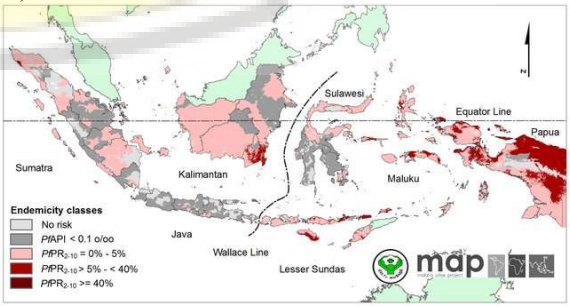

Fig. 1. Endemicity class predictions of *Plasmodium falciparum* malaria PfPR2–10 in Indonesia [8].

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 antimetabolic medicament by the indigenous people of Indonesia including antidiabetic, 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.

Fig. 2. From left to right, flowering plants of *Swietenia mahagoni* and *Kibatalia arborea*.

II. METHODS

A. Sample collection

Mature fruits of *Swietenia mahagoni* (L.) Jacq. (Meliaceae) were collected from Klaten-Central Java. The seeds were obtained ad dried under shades. Voucher samples were also transported to the Faculty of Pharmacy-University of Jember for storing and identification under accession number SMS. Leaves of *Kibatalia arborea* (BI.) G. Don. (Apocynaceae) was obtained from Materia Medika Batu. Voucher sample was store at the Faculty of Pharmacy-University of Jember under accession number KAL.

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-H_2SO_4 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. ^{1}H-NMR study

Each sample (5 mg) was dried through a high vacuum apparatus under the silica gel chamber before dissolving in CD_3OD. The ^{1}H-NMR was recorded in Jeol NMR 400 MHz.

D. Anti-plasmodium bioassay

Each sample (1 mg) was dissolved in DMSO (100 uL) 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 uL) with serial concentration were loaded into 96 well plate followed by parasite (198 uL) addition to form last concentration as 100, 10, 1, 0.1 and 0.01 ug/mL. The plate was treated with mixed gases (5% O_2, 5% CO_2 and 90% N_2) prior incubation at 37°C. The culture was collected, stripped and stained with 10% Giemsa reagent. Percentage inhibition was calculated based on formula 1.

$$\% \text{ Inhibition} = 100\% - ((Xu/Xk) \times 100\%) \quad (1)$$

Note Xu=% growth of sample, Xk=%growth of the negative control. IC_{50} 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,* swiemahogins 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-trimethoxy4-methyl-1,2,3,4-tetrahydro-isoquinoline 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.

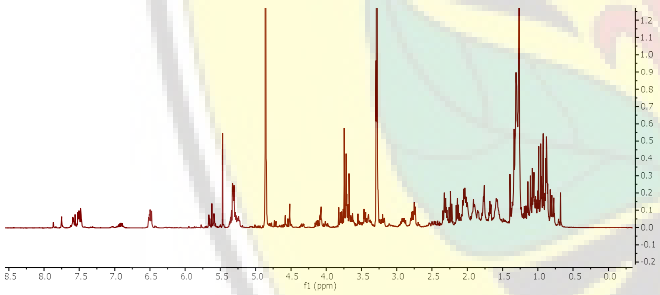

Fig. 4. ^{1}H-NMR spectrum of crude methanol extract of leaves of *Swietenia mahagoni*.

Literature-based dereplication revealed no significant report regarding alkaloid produced from the seed of *S. mahagoni*. Moreover, there was no report regarding the anti-plasmodium activity of the seeds of *S. mahagoni* and very limited data on antimalarial bioprospecting on closed species *S. macrophylla*. In this study, the crude methanol of seed of *S. mahagoni* exhibited 100% Plasmodium growth inhibition at 100 µg/mL with IC_{50} value of 7.40 µg/mL.

Locally named as *kayu santen*, *Kibatalia arborea* has been confused about its closed tree, *Kopsia arborea*. The phytochemical screening study clearly indicated the presence of less polar alkaloids based on the Dragendorff test. Phenolic components were also clearly identified through vanillin reagent-based test results. The HPLC chromatogram (Fig. 5) also successfully distinguish of possible polar phenolic constituents and less polar constituents in which the last could be a representative of alkaloid chemotypes.

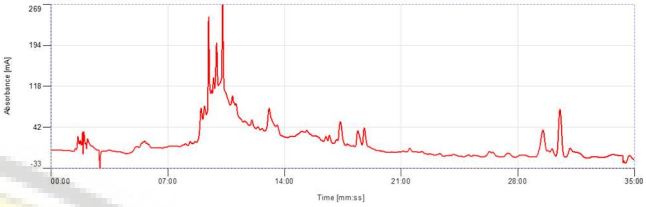

Fig. 5. HPLC profile of crude methanol extract of leaves of *Kibatalia arborea* obtained from 10-90% acetonitrile in water development recorded at 254 nm.

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].

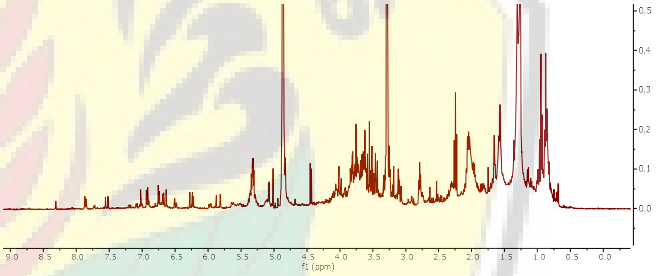

Fig. 6. ^{1}H-NMR spectrum of crude methanol extract of leaves of *Kibatalia arborea.*

This study reported the antimalarial activity of Kibatalia species, in which crude methanol extract of leaves of *Kibatalai 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.

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