Searching for antimalarial agent from Indonesian *Combretum indicum* and *Magnolia figo*

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***Abstract*- The archipelagic country of Indonesia has been an endemic area of malaria in which the Indigenous people of Indonesia have used medicinal plants to fight against plasmodial parasites. The study focused on two medicinal plants of Indonesia, namely *Combretum indicum* and *Magnolia figo.* Phytochemical, spectroscopic, and bioactivity assay protocols were performed. The experiments resulted in the major components detected were terpenoids and phenolic constituents. The bioassay indicated significant antimalarial potency of the crude methanol extract of leaves of *Combretum indicum* and *Magnolia figo*.**

Keywords—Combretum indicum; Magnolia figo; Malaria; Plasmodium falciparum

I. INTRODUCTION

Indonesia has been one of the malarial endemic countries in South East Asia with an estimated number of cases of 1.2 million in 2017 [1]. More than 25 Anopheles species infested Indonesia were malarial vector. Indonesia reported a significant improvement in malaria control [2]. More than 50% districts in Indonesia were free from malaria in which left 72% population inhabited malarial free-region. Nevertheless, malarial endemicity is projected to remain high in Eastern Indonesia (Fig. 1).

Malaria involves infective parasites *Plasmodium falciparum, P. vivax, P. malariae, P. ovale* in which *P. falciparum* is responsible for the highest mortality [3]–[6]. New malarial parasite *Plasmodium knowlesi* were firstly reported from South East Asia [3]–[6]. In 2016, 216 million malarial cases was reported globally which 14.6 million cases was in South East Asia [6]. Antimalarial resistant Plasmodium has made malaria control and eradication to become more complicated. Falciparum malaria superbug occured in Cambodia poses a serious threat [7], [8].

The majority of antimalarial agents have been derived from natural products obtained from medicinal plants. The first antimalarial, quinine, was obtained from the bark of cinchona tree, a native tree from Southern America [9], [10]. Bioprospecting study on Indonesian medicinal plants used by the indigenous people of Indonesia in malarial fever therapy have revealed significant results (Fig. 2). This exploration was successfully isolated samaradine Y from *Quassia indica* with IC_{50} value of 0.014 μM [11]. This compound was more active than a standard malarial drug, chloroquine with IC_{50} value of 0.29 μM [12].

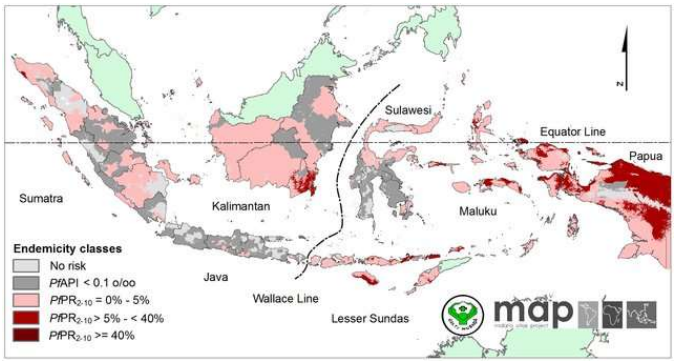

Fig. 1. Endemicity class predictions of *Plasmodium falciparum* malaria PfPR2–10 in Indonesia [13]

Fig. 2. Location of previous bioprospecting study to reveal antimalarial constituents from endemic medicinal plants [12]

In this study, two medicinal plants of Indonesia (*Combretum indicum* and *Magnolia figo*, Fig. 3) were evaluated for their phytochemical constituents and pharmacological activity against malarial parasite. *Combretum indicum* is a shrub and well distributed across the archipelago of Indonesia where the species pronounced as "wudani" by people in Sumatra, *bidani* by Sundanesse, *ceguk* by Javanesse, *rabet dani* by Maduresse, *tigao* by Buginese, and *kunyi rhabet* by the Indigenous people of small Sunda Islands [14]. The seeds were traditionally prepared as an anthelmintic agent. *Magnolia figo* was one of famous flower producing plant in Indonesia which is locally named as *cempaka*. The leaves are traditionally used in malarial

fever therapy, whereas the flowers are commonly used as analgesic to treat headache [15].

Fig. 3. From left to right, *Combretum indicum* and *Magnolia figo* tress at flowering stages

II. METHODS

A. Sample and extraction

Medicinal plant samples, leaves of *Magnolia figo* (Lour.) DC. (Magnoliaceae) and *Combretum indicum* (L.) C.C.H. Jongkind (Combretaceae) were obtained from Materia Medika Batu, Malang, East Java as dried leaves in which the samples were then stored and labelled under accession number M-20 and PI, respectively. Each dried sample (1 g) was grinded and extracted with methanol (10 mL), and sonicated for 1 hour. Supernatant was collected and dried.

B. Phytochemical study

Crude methanol extract was developed in a Thin Layer Chromatography (TLC) plate using dichloromethane as developing solvent. Vanillin-H_2SO_4 staining agent was deployed to visualize the chemotype which red color indicated for phenols present, grey for sugar, purple for terpenoids. Alkaloid present was detected using Dragendorff's reagent with red-orange color.

C. ^{1}H-NMR study

A portion of sample (5 mg) was completely dried through high vacuum apparatus under silica gel chamber. Dried sample was dissolved in CD_3OD and ^{1}H-NMR was recorded in Jeol NMR 400 MHz.

D. Anti-plasmodium bioassay

Sample (1 mg) was dissolved in DMSO (100 uL) and a serial dilution was performed to gain 1000, 100, 10, 1 ug/mL Parasite used in the study was *Plasmodium falciparum* 3D7 strain at ring stadium with parasitemia level of ± 1%. Crude extract sample (2 uL) at concentration series was loaded into 96 well plate. Parasite (198 uL) was added in which the last concentration as 100, 10, 1, 0.1 and 0.01 ug/mL. The plate was then allocated into a chamber with mixed gass (5% O_2, 5% CO_2 and 90% N_2) prior incubation at 37°C. The culture was then collected, stripped and stained with 10% giemsa reagent. Percentage inhibition was calculated based on formula below:

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

Note: Xu=% growth of sample, Xk=%growth of negative control. IC_{50} was obtained as concentration which inhibit 50% growth of the parasite.

III. RESULTS AND DISCUSSION

As part of antimalarial project, this paper considered reporting two species, namely *Combretum indicum* and *Magnolia figo*. Among two species in this study, the Indonesian medicinal plant inventory indicated only leaves of *Combretum indicum* to constitute whereas the sample in this study revealed no alkaloid was detected. The Dragendorff remains versatile alkaloid detecting reagent with limit detection as less as 0.1 ppm. Therefore, the antimalarial activity was suggested from non-alkaloid content in which TLC-based analysis using vanillin reagent figured the crude methanol extract of the leaves of *Combretum indicum* producing a distinct purple color at less polar and orange at polar retention regions. These are a clear indication that the leaves constitute terpenoid and phenolic components. The High-Pressure Liquid Chromatography (HPLC) profile of the crude extract (Fig. 4) showed at least major components with UV chromophore containing compounds.

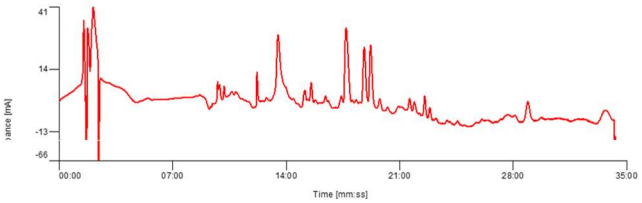

Fig. 4. HPLC profile of crude methanol extract of leaves of *Combretum indicum* obtained from 10-90% acetonitrile in water development recorded at 254 nm

A sensitive malarial parasite, *Plasmodium falciparum* 3D7 was grown under crude methanol extract in which 100% inhibition was obtained at 100 µg/mL concentration producing a significant IC_{50} value of 4.12 µg/mL. Further extensive isolation work is necessary to reveal the major and minor constituents of the crude methanol extract.

Although *Combretum indicum* is the accepted name, its synonym *Quisqualis indica* was found in several publications revealed it constituents including the isolation of a volatile alkaloid quinoline-4-carbonitril from flower. The alkaloid was reported to possess a significant anti-inflammatory and antioxidant activities [16]. Anticancer active components was previously recorded from the leaves, 25-O-acetyl-23,24-dihydro-cucurbitacin F [17]. The effectiveness of *Combretum indicum* in controlling malarial vector was previously reported with no anti-plasmodial activity found [18].

Dragendorff treatment showed no orange color, suggesting no nitrogen containing compounds was present in the crude methanol extract. Based on phytochemical profiling using vanillin reagents, the crude methanol extract of *Magnolia figo* clearly indicated the presence of terpenoids based on deep purple color. Glycoside was a minor chemotype components as

no strong grey spot was produced under the same protocol. Reddish color on the test showed the presence of phenolic type compounds. The HPLC chromatogram (Fig. 5) showed major peaks existed in retention time below 50% acetonitrile in water indicated the high presentation of phenolic constituents.

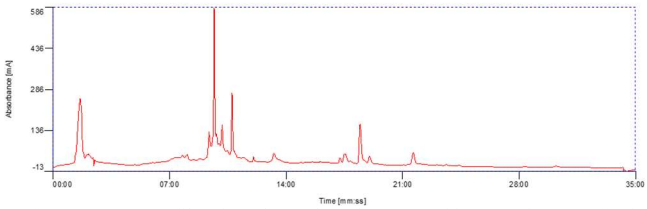

Fig. 5. HPLC profile of crude methanol extract of leaves of *Magnolia figo* obtained from 10-90% acetonitrile in water development recorded at 254 nm

Proton NMR spectrum (Fig. 6) analysis on the crude methanol extract indicated peaks at 7 ppm for aromatic protons, sugar components were represented by peaks at around 3-5 ppm and terpenoid constituents were represented by peaks at around 0.5-2 ppm.

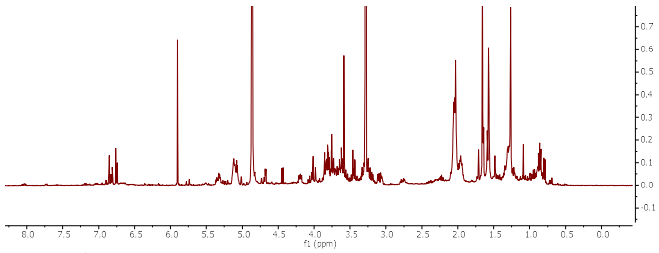

Fig. 6. ^{1}H-NMR spectrum of crude methanol extract of leaves of *Magnolia figo*

Anti-plasmodial bioassay revealed the crude methanol extract of *Magnolia figo* has a significant activity with IC_{50} value of 13.42 µg/mL.

Previous research on this species were commonly reported under *Michelia figo*. The leaves from Taiwan was previously reported to constitute a sesquiterpene lactone (11,13-dehydrolanuginolide), alkaloid ((–)-nuciferine, (–)-anonaine, and N-methylcorydaldine), steroids (*β*-sitostenone, stigmasta-4,22-dien-3-one), benzenoids (*p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, methylparaben, vanillin), chlorophylls (pheophytin a, pheophorbide a, pheophytin b, pheophorbide b, aristophyll-C, 132-hydroxy-(132-S)- pheophytin a) [19]. However, information regarding their antimalarial were limited. Bisbenzylisoquinoline alkaloid magnolin (Fig. 7) from leaves of Thai *M. figo* were previously reported to possess anti-malarial activities against both sensitive *Plasmodium falciparum* FCR3 and resistant *Plasmodium falciparum* K1 strains with IC_{50} value of 0.16 and 1.51 µM, respectively [20].

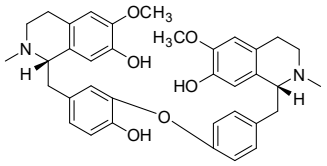

Fig. 7. Antimalarial agents, magnoline from Thai *M. figo* [20]

Compared to the previously studied samples, the Indonesian origin samples in this study did not show alkaloid components, which suggest other chemotype migh contribute to the claims.

IV. Conclusions

The study revealed the antimalarial potency of the crude methanol extract of *Combretum indicum* and *Magnolia figo* with significant activity against *Plasmodium falciparum* 3D7. Further research is necessary to reveal the chemotypic constituents which responsible for the claims. Nevertheless, these validated the traditional claims of antimalarial medicinal plants used by the indigenous people of Indonesia.

Acknowledgment

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

References

[1] World Health Organization, *Malaria country profile: Indonesia*, 2017.
[2] V. Sitohang *et al.*, "Malaria elimination in Indonesia : halfway there," *Lancet Glob. Heal.*, vol. 6, no. 6, pp. e604–e606, 2018.
[3] N. J. White, "Plasmodium knowlesi : the fifth human malaria parasite," *Clin. Infect. Dis.*, vol. 46, pp. 172–173, 2008.
[4] J. Cox-Singh and B. Singh, "Europe PMC Funders Group Knowlesi malaria : newly emergent and of public health importance ?," *Trends Parasitol.*, vol. 24, no. 9, pp. 406–410, 2008.
[5] B. E. Barber, G. S. Rajahram, M. J. Grigg, T. William, and N. M. Anstey, "World Malaria Report : time to acknowledge Plasmodium knowlesi malaria," *Malar. J.*, vol. 16, no. 135, 2017.
[6] World Health Organization, *World malaria report 2017*, Geneva: World Health Organization, 2017.
[7] M. Imwong, T. T. Hien, N. T. Thuy-Nhien, A. M. Dondorp, and N. J. White, "Spread of a single multidrug resistant malaria parasite lineage (PfPailin) to Vietnam," *Lancet Infect. Dis.*, vol. 17, no. 10, pp. 1022–1023, 2017.
[8] T. John, "The 'super-malaria' on the rise in Southeast Asia," *TIME*, 2017.
[9] J. Achan *et al.*, "Quinine, an old anti-malarial drug in a modern world: role in the treatment of malaria," *Malar. J.*, vol. 10, no. 1, p. 144, 2011.
[10] K. J. Arrow, C. Panosian, and H. Gelband, *Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance Kenneth*. Washington DC: The National Academies Press, 2004.
[11] I. Kitagawa *et al.*, "Indonesian medicinal plants. XVII. Characterization of quassinoids from the stems of Quassia indica.," *Chem. Pharm. Bull. (Tokyo).*, vol. 44, no. 11, pp. 2009–2014, 1996.
[12] A. S. Nugraha and P. a. Keller, "Revealing indigenous Indonesian traditional medicine: Anti-infective agents," *Nat. Prod. Commun.*, vol. 6, no. 12, pp. 1953–1966, 2011.
[13] I. Elyazar *et al.*, "Plasmodium falciparum Malaria Endemicity in Indonesia in 2010," *PLoS One*, vol. 6, p. e21315, Jun. 2011.
[14] J. R. Hutapea, *Inventaris tanaman obat Indonesia I jilid 1*. Jakarta: Departemen Kesehatan dan Kesejahteraan Sosial RI, 2000.

[15] Djumidi, *Inventaris tanaman obat Indonesia IV*. Jakarta: Departemen Kesehatan RI, 1997.
[16] P. K. Rout *et al.*, "A quinoline alkaloid rich *Quisqualis indica* floral extract enhances the bioactivity," *Nat. Prod. Res.*, pp. 1–7, Jul. 2019.
[17] B. Lohberger *et al.*, "25-O-acetyl-23,24-dihydro-cucurbitacin F induces cell cycle G2/M arrest and apoptosis in human soft tissue sarcoma cells," *J. Ethnopharmacol.*, 2015.
[18] M. Govindarajan, P. Vijayan, S. Kadaikunnan, N. S. Alharbi, and G. Benelli, "One-pot biogenic fabrication of silver nanocrystals using Quisqualis indica: Effectiveness on malaria and Zika virus mosquito vectors, and impact on non-target aquatic organisms," *J. Photochem. Photobiol. B Biol.*, 2016.
[19] H. C. Chen, C. L. Kao, C. T. Chen, H. T. Li, and C. Y. Chen, "Chemical Constituents of the Leaves of Michelia figo," *Chem. Nat. Compd.*, vol. 54, no. 2, pp. 407–410, Mar. 2018.
[20] A. Phrutivorapongkul *et al.*, "Anti-plasmodial activity of bisbenzylisoquinoline alkaloids from Michelia figo leaves.," *Thai J. Heal. Res.*, vol. 20, no. 2, pp. 121–128, 2006.