Searching for antimalarial agent from Indonesian

**Combretum indicum** and **Magnolia figo**

Ari Satia Nugraha\(^1\), Dwi Koko Pratoko\(^1\), Bawon Triatmoko\(^1\), Antonius Nugraha Widhi Pratama\(^1\), Wilda Nur Rohmatillah\(^1\) and Tinton Agung Laksono\(^1\)

\(^1\)Drug Utilisation and Discovery Research Group, Faculty of Pharmacy, University of Jember, Jember, 68121, Indonesia

\(^*\)E-mail: arisatia@unej.ac.id

**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 \(\mu\)M [11]. This compound was more active than a standard malarial drug, chloroquine with IC\(_{50}\) value of 0.29 \(\mu\)M [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$_2$SO$_4$ 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 no nitrogen containing compounds was present in the crude methanol extract. Based on phytochemical profiling using 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.

![HPLC profile of crude methanol extract of leaves of Combretum indicum obtained from 10-90% acetonitrile in water development recorded at 254 nm](image)

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 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.

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.

Note: X$_u$=% growth of sample, X$_k$=%growth of negative control. IC$_{50}$ was obtained as concentration which inhibit 50% growth of the parasite.

$$\text{% Inhibition} = 100\% - ((X_u/X_k) \times 100\%) \quad (1)$$
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.

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.

Anti-plasmodial bioassay revealed the crude methanol extract of Magnolia figo has a significant activity with IC50 value of 13.42 µg/mL.

Previous research on this species were commonly reported to constitute a sesquiterpene lactone (11,13-dihydrolanuginolide), 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 antimalarial activities against both sensitive Plasmodium falciparum FCR3 and resistant Plasmodium falciparum K1 strains with IC50 value of 0.16 and 1.51 µM, respectively [20].

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.

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REFERENCES


