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Senna occidentalis and *Cyanthillium patulum*: Indonesian Herbs as Source for Antimalarial Agents

Antonius Nugraha Widhi Pratama[1], Dwi Koko Pratoko[1], Bawon Triatmoko[1], Naura Bathari Winarto[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*- As stated in history, antimalarial agents are closely related to natural product research. *Senna occidentalis* and *Cyanthillium patulum* are among many potential medicinal plants based on ethno-traditional knowledge. This study was conducted to profile the activity for antimalarial from the crude methanol extract of the plant, which were collected from Malang,-East Java and Klaten-Central Java, Indonesia. Antimalarial assay against *Plasmodium falciparum* was done *in vitro*. Chemical identification using Dragendorff's reagent and spectral analysis from ^{1}H-NMR spectroscopy was conducted. The results demonstrate that the extract of leaves of *S. occidentalis* constitutes typical phenolic backbones whereas *C. patulum* constitutes sugar derivatives and terpenoids. Crude methanol extracts of the two species possessed significant antimalarial activity. The further experiment is required to define the responsible antimalarial compounds.**

Keywords*— *Indonesian medicinal plant; Antimalaria; Senna occidentalis; Cyanthillium patulum

I. INTRODUCTION

Malaria has remained a significant public health problem especially for those who live in the tropical and subtropical regions of the world, including Indonesia. It has been roaming in the human population since estimated between 10,000 and 100,000 years ago, starting from Africa [1]. This infectious disease is caused by a mosquito bite that injects *Plasmodium* parasites into the human bloodstream. By the recognition of *Plasmodium knowlesi* in 2017 as a new cause of malaria, there are five species of Plasmodium known infectious to humans [2-4]. Among them, *P falciparum* remains the most important cause due to its morbidity and mortality.

The World Health Organization's (WHO) latest report on malaria estimates that in 2017 219 million people worldwide were infected with malaria (95% confidence interval [CI] 203-262 million). The estimate of global malarial deaths in 2017 was 435 thousand, of which 61% were under 5 children, the most vulnerable group [5]. Malaria prevention and control programs had reached pleasant achievement during 2000 and 2015 by the significant reduction of cases and deaths, but the development has been halted since then [6]. This could hamper the 2030 goal to reduce malaria incidence and mortality by up to 90% [7].

Several problems that have emerged interfering with malaria prevention and control programs include lower efficacy of the vaccine, resistance to the insecticide used for a bed net, and resistance to available antimalarials [7-8]. One vaccine candidate (the RTS, S/AS01) that underwent a phase III clinical trial in seven Sub-Saharan African countries did not show ample protection for children aged 5-17 months and even younger [9-10]. Nine more candidates are in progress spreading from preclinical to phase II clinical trials [11]. Moreover, the use of pyrethroid-treated bed nets has been an essential part of malaria control and therefore the resistance to them has attracted serious attention [12-13]. Even though a Cochrane systematic review strongly suggests that the use of the insecticide-treated bed net has remained beneficial compared to the nonet and not treated net in terms of *P falciparum* mortality and prevalence, this situation causes growing apprehension [13]. Furthermore, resistance to antimalarials, which commonly first occurred in Southeast Asian countries, also contributes to the problems for malaria control. As summarized by Menard and Dondorp [14], the first partial artemisinin resistance to *P falciparum* was identified in Cambodia and the first resistance to artemisinin and its partner drugs also occurred in Cambodia a Thai-Myanmar border. The resistance of *P. vivax* to chloroquine was emerging in Indonesia. All of these problems pushed the need for finding new antimalarials. In addition endemicity projection of malaria in the archipelago request serious attention, especially in the East-Indonesia (Fig. 1).

Although not all antimalarials are derived from natural products, the discovery of antimalarials cannot be separated from them. The first antimalarial quinine was first isolated by a European scientist in 1820 from *Chincona officinalis* L. that grew wild in South America and was cultivated in Java [15], [16]. Other antimalarial candidates were taken from *Dichroa febrifuga* L. and *Brucea javanica* (L.) M. by Chinese scientists, but they did not reach clinical development phase [15]. In the dawn of resistance to quinine and its derivatives, a Chinese scientist first isolated artemisinin from *Artemisia annua* in 1971 [15-16]. Until now, artemisinin and its derivatives have been used with other antimalarials in artemisinin-based combination therapy (ACT) as the first line to treat multi-drug resistant falciparum malaria.

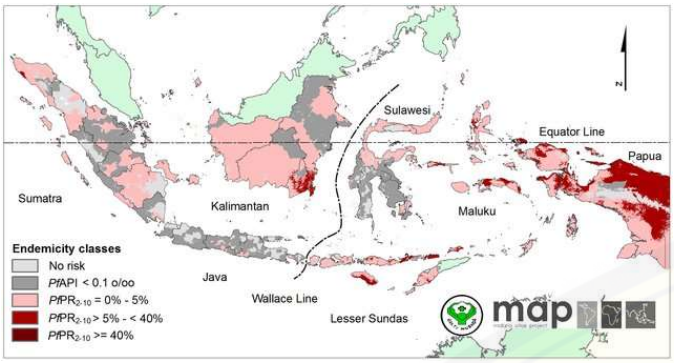

Fig. 1. Endemicity class predictions of *Plasmodium falciparum* malaria PfPR2–10 in Indonesia [17].

In this study, two Indonesian medicinal plants, *Senna occidentalis* and *Cyanthillium patulum* (Fig. 2), were evaluated for their phytochemical, spectral and biological activity against a sensitive strain, *Plasmodium falciparum* 3D7. *Senna occidentalis* (L.) Link is distributed in the archipelagic of Indonesia and known as *kopi andelan* in Sumatra Island, *menting* by Javanese people, *kasingsat* by Sundanese people and *balinggang alas* by the Indigenous people of lesser Sunda Island. The leaves have been prepared traditionally to treat infective diseases caused by *Plasmodium species* and *Sarcoptes scabiei* parasite [18]. The mixture of the leaves and seeds was prepared as laxative agents [19]. *Cyanthillium patulum* herb was commonly named as *selentrong* in Central Java in which the whole plant has been prepared to treat diarrhea. This plant has similar uses as the closed species, *C. cinerea* Less. to treat diarrhea, headache, convulsion, stomach-ache, and cough [20].

Fig. 2. From left to right, ***Senna occidentalis*** *and* ***Cyanthillium patulum*** at flowering stages.

II. METHODS

A. Sample collection

The leaves and seeds of *Senna occidentalis* (L.) Link (Leguminosae) and the whole plant of *Cyanthillium patulum* (Dryand ex Ait) H. Rob. (Asteraceae) were collected from Klaten, Central Java in July 2019. The sample was brought to the Faculty of Pharmacy, University of Jember, East Java for preparation and identification. Voucher sample was kept in the Faculty under accession number SOL, SOS, and CP respectively.

B. Extraction

Air-dried sample (1 g) was pulverized and soaked with methanol (10 mL) and sonicated for 1-hour prior filtration. The filtrate was collected and dried to gain crude methanol extract.

C. Phytochemical study

Phenolic, glicoside and terpenoid content were detected through Thin Layer Chromatographic (TLC) method based on Vanillin-H_2SO_4 visualizing reagent in which the constituents were indicated as red, grey and purple color. Alkaloid chemotype was detected by Dragendorff reagent with orange color as a positive indication.

D. ^{1}H-NMR study

The crude methanol extract was vacuum dried under the silica gel chamber. Dried sample (5 mg) was dissolved in CD_3OD and was then loaded into Jeol NMR 400 MHz to record ^{the} ^{1}H-NMR spectrum.

E. Anti-plasmodium bioassay

A stock solution of the sample was prepared by dissolving crude extract (1 mg) with 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%. Sample at concentration series (2 uL) was loaded into 96 well plates. Parasite (198 uL) was added with final of 100, 10, 1, 0.1 and 0.01 ug/mL. The well-plate was treated with mixed gases (5% O_2, 5% CO_2 and 90% N_2) followed by incubation at 37°C. The culture was harvested, stripped and stained with 10% Giemsa reagent. Percentage inhibition was calculated based on formula 1 below:

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

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

Phytochemical screening on the crude methanol extract of leaves of *S. occidentalis* showed intense purple color which indicated the presence of terpenoid constituents. The phenolic presentation was shown by red color in the polar region with minimum retention time. This agreed with HPLC chromatogram data showing major peaks existing at short retention time (Fig. 2, above). Phytosterols are very commonly existed in leaves. Compared to the leaves, the seeds extract constituted a wider range polarity of secondary metabolites as shown in the HPLC chromatogram profile (Fig. 2, below). Only

seed methanol extract produced orange color based on Dragendorff's test which indicated alkaloid presence.

HPLC chromatogram (Fig. 3) revealed the leaves to constitute typical phenolic components in which the proton spectrum of crude methanol extract suggested a kaempferol fingerprint at the aromatic region (Fig. 4).

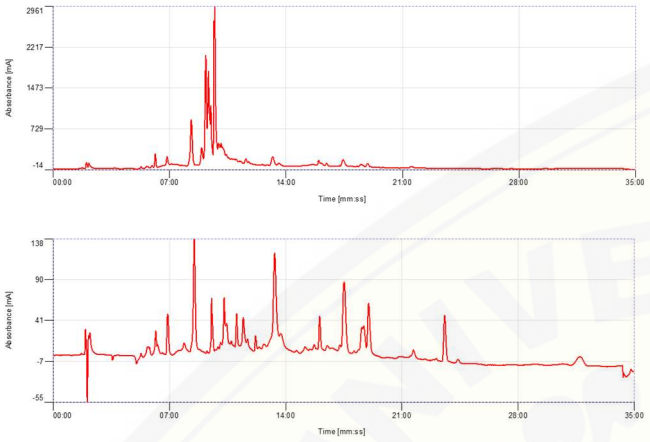

Fig. 3. HPLC profile of crude methanol extract of leaves of *S. occidentalis* (top) and seeds of *S. occidentalis* (bottom). The profiles were obtained from 10-90% acetonitrile in water development recorded at 254 nm.

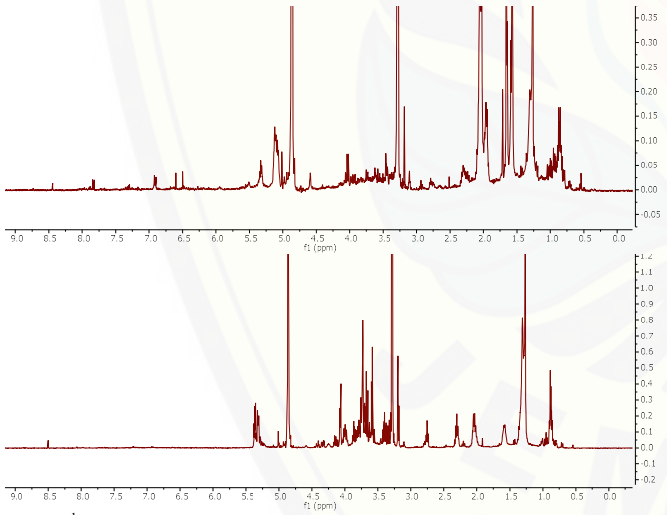

Fig. 4. ^{1}H-NMR spectrum of crude methanol extract of leaves (top) and seeds (bottom) of *S. occidentalis*.

The previous study on the leaves of the same species revealed a different type of phenolic compounds, including flavonoid and anthraquinone compounds. Sugars were also reported from the seeds of *S. occidentalis* [21]. However, no alkaloid was previously reported from the seeds of *S. occidentalis*.

The crude methanol extract of the leaves and seeds against *Plasmodium falciparum* 3D7 showed significant activity with an IC_{50} value of 12.19 µg/mL and 6.82 µg/mL, respectively. The previous study on the same species revealed the potency of the crude extract of leaves of *S. occidentalis* as malaria vector repellant and antimalarial agents against chloroquine sensitive plasmodium with an IC_{50} value of 48.80 µg/mL [22]. Six quinones from the leaves of *S. occidentalis* were previously reported to possess significant anti-malaria against *Plasmodium falciparum* [23]. There has been no report regarding the anti-malarial activity of the seeds in which in this study, the seeds which constitute alkaloid possessed higher activity compared to the leaves.

The phytochemical screening of the leaves of *Cyanthillium patulum* resulted in no nitrogen-containing compound that was detected based on the negative Dragendorff reagent test. On the other hand, the vanillin based reagent test indicated the crude methanol extract of *C. patulum* to possess highly sugar derivative contents based on the presence of grey color. This agreed with proton NMR spectral analysis of the crude methanol extract of *C. patulum* (Fig. 6).

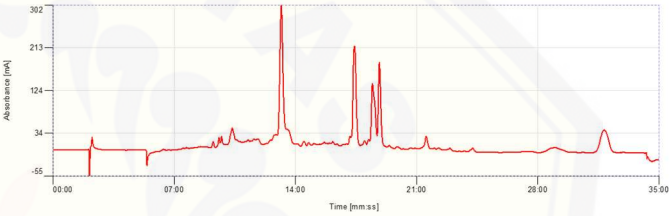

Fig. 5. HPLC profile of crude methanol extract of leaves of *C. patulum*. The profiles were obtained from 10-90% acetonitrile in water development recorded at 254 nm.

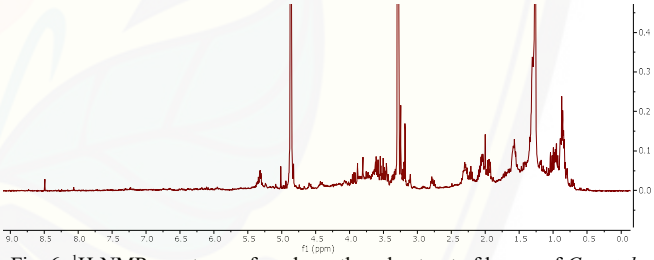

Fig. 6. ^{1}H-NMR spectrum of crude methanol extract of leaves of *C. patulum*.

The antimalarial activity of the crude extract of leaves of *C. patulum* indicated a significant anti-malarial activity against sensitive *Plasmodium falciparum* 3D7 with an IC_{50} value of 4.45 µg/mL.

IV. Conclusion

The phytochemical and pharmacological studies of *S. occidentalis* revealed significant antimalarial activity in which limited records on its alkaloid constituents. Whereas, crude methanol extract of the leaves of *C. patulum* indicated a high potential for antimalarial agent source in which no previous phytochemical and pharmacological records.

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